MAR 26 1917
UNIV. OF MICH.
LIBRARY

Vol. II MARCH, 1917 No. 6

The Journal of Laboratory and Clinical Medicine

VICTOR C. VAUGHAN, M.D., Editor-in-Chief
University of Michigan, Ann Arbor

ASSOCIATE EDITORS

Pharmacology
DENNIS E. JACKSON, M.D.
Washington University, St. Louis

Bacteriology
HANS ZINSSER, M.D.
Columbia University, New York

Immunology and Serology
FREDERICK P. GAY, M.D.
University of California, Berkeley

Physiological Pathology
PAUL G. WOOLLEY, M.D.
University of Cincinnati, Cincinnati

Physiological Chemistry and Clinical Physiology
J. J. R. MacLEOD, M.B.
Western Reserve University, Cleveland

ROY G. PEARCE, M.D.
Lakeside Hospital, Cleveland

Clinical Microscopy and Laboratory Technic
ROGER S. MORRIS, M.D.
University of Cincinnati, Cincinnati

Tuberculosis
GERALD B. WEBB, M.D.
Cragmor Sanatorium, Colorado Springs

Published by
THE C. V. MOSBY COMPANY
ST. LOUIS

$3.00 Per Annum Single Copies, 30 Cents

The Handy Ampoule.

Our Sterilized Solutions for Hypodermatic Injection Have Solved a Difficult Problem.

FORMERLY when a physician wished to resort to hypodermatic medication he had to use tinctures, fluid extracts or solutions of his own making. Often his solutions were found to contain precipitates. Frequently sterile water was not to be had when wanted. Result: delay and disappointment—sometimes even risk of life.

Parke, Davis & Co.'s Sterilized Solutions in Ampoules have cleared away the difficulties.

ADVANTAGES.

1. Solutions in ampoules are always ready for use.

2. They are sterile.

3. The dose is accurate, a definite amount of medicament being contained in each milliliter (Cc.) of solution.

4. The drug is treated with the most suitable solvent—distilled water, physiologic salt solution, or oil, as the case may be.

5. The container is hermetically sealed, preventing bacterial contamination.

6. An impervious cardboard carton protects the solution against the actinic effect of light.

◆ ◆ ◆

We supply upward of sixty ready-to-use sterilized solutions. They are described in our catalogue, under "Ampoules," pages 194-200. Consult this valuable list.

OUR "AMPOULES" BROCHURE

gives therapeutic suggestions, descriptions of packages, prices, etc. Every physician should have this book. We send it, postpaid, on receipt of request.

Home Offices and Laboratories,
Detroit, Michigan.

Parke, Davis & Co.

50 Years of Pharmaceutical Progress

The Journal of Laboratory and Clinical Medicine

VOL. II. ST. LOUIS, MARCH, 1917 NO. 6.

ORIGINAL ARTICLES

SPIROCHÆTES*

BY HIDEYO NOGUCHI, M.D., NEW YORK CITY.

TODAY a spirally shaped microorganism may be called either Spirochæta, Spirillum, Treponema, Spironema, Cristispira, or Saprospira, according to the characteristics of the organism. The choice of the generic name for a given variety is still very much dependent upon the individual views held by different investigators, and this has led to a somewhat chaotic state of affairs in the nomenclature of this group of organisms. This is found to be the case more especially in the medical literature where these minute spiral organisms play an important part as causative agents of certain diseases. Nevertheless, thus far but little attention has been paid to the systematic position occupied by them. Since Ehrenberg,[1] in 1838 introduced a new generic term "Spirochæta" to designate a free living spiral organism which he found in a swamp near Berlin, it remained practically unnoticed until 1904 when Schaudinn[2] stated as his view that so-called Spirochæta constitutes a phase of the life cycle of trypanosomes; hence, that they are of protozoan origin instead of being plants. It may here be mentioned that Ehrenberg, Migula, and other systematists classified the Spirochete under Bacteria, which classification was accepted for nearly seventy years. Indeed, it was not uncommon among medical authorities to employ the terms Spirochæta and Spirillum interchangeably. Medical men may consider the causative agent of relapsing fever as being a Spirillum or a Spirochæta, according to their inclination. This sort of indiscriminate use of terms has gradually extended to other spiral organisms, such as the causative agent of syphilis. According to the old school it was of very little importance whether a spiral organism had one or two polar flagella or a tuft of flagella, so long as both Spirochæta and Spirillum belonged to the same family. On the other hand, Schaudinn and his school maintained that the difference between Spirochæta and Spirillum is no longer so

*From the Rockefeller Institute for Medical Research.
Harvey lecture delivered on February 5, 1916, at the New York Academy of Medicine.

easily disposed of since one is of plant and the other of animal origin. The revolutionary view of Schaudinn was based chiefly upon his observations on a protozoon. *Leucocytozoon ziemanni* is regarded by Schaudinn as a trypanosome found in the blood of the little owl (Athene noctua), which is said to undergo a spirochætal stage while passing through an intermediary host. While the accuracy of Schaudinn's observations has been questioned by later investigators,[3, 4, 5] the great impetus which his theory occasioned has had a far-reaching effect upon the development of our present knowledge concerning the organisms generally known as "spirochætes." It was soon after announcing his views that Schaudinn made his famous discovery of the occurrence of *Spirochæta pallida* in syphilis. In their first publication Schaudinn and Hoffmann[6] gave the name "*Spirochæta pallida*" to the spiral organism found in syphilitic lesions because of its resemblance to Spirochætes in general, but within a year Schaudinn recognized certain features (preformed cylindrical spiral filament, difficulty in staining, regularity of curves, etc.), which he considered distinctive enough to classify it apart from the usual Spirochætes (changeable curves, taking on of a violet component of Giemsa, no preformed spiral, ribbon form, etc.).[7, 8] Thereupon he replaced the generic name Spirochæta with a new term Treponema.[9] This all occurred in 1905. But before Schaudinn had had time to decide upon a new generic name for his organism, Vuillemin[10] (1905) proposed that it be called "Spironema." In the meanwhile some authors, particularly in France, commenced to use the term "Spirille." There were also some newer generic names created by still later systematists, such as Microspironema[11] (Stiles and Pfender*), Borrelia[12] (Swellengrebel), Spiroschaudinnia[13] (Sambon), and Spirosoma[14] (Schilling), but these are of no importance today. The only difficulty in choosing the generic name for "Spirochæta pallida," lies in the fact that although Schaudinn corrected his error within several months after his discovery, another suggestion had meanwhile been made to answer the same purpose, and according to the international code of nomenclature, Vuillemin's Spironema would have had to receive preference over Schaudinn's own Treponema, had it not been for the fact that the term Spironema as proposed by Vuillemin is not acceptable to those who maintain, like Schaudinn, that the organism of syphilis belongs to the protozoa, because in 1892 it was used by Klebs as a genus of Flagellate.[15] The same name had also been used by Meek in 1864 for a fossil snail. Of course, "Spironema" may be available for any one who holds that "spirochetes" do not belong to Protozoa.† Thus, Gross[16] (1910) used this term to include various spirochætes allied to the spirochætes of relapsing fevers, syphilis, etc., with the specification that he believed these to be of bacterial nature. It may be mentioned that the term "Spirochæta," as taken up by Schaudinn in 1905 in the sense of protozoan organism, had already been used by Michael Sars in 1856 for an annilid genus. It seems that the creation by Schaudinn of the genus Treponema was perfectly justified, although not all the characteristics attributed by him to this genus are found to be distinctive from those of other "spirochætes." Schau-

*The statement of some authors (Gross and Gonder) that this antedated Schaudinn is erroneous. Schaudinn published his note on October 19, and Stiles and Pfender on December 2 of the same year.

†The use of two identical terms, one in the animal and the other in the plant kingdom, has been known to occur and is permissible. For example, "Bacillus" and "Coccus" are found in zoological as well as in botanical genera.

dinn did not live long enough to witness the gradual modification which the Spirochæta question went through. As a result of the works of various systematists and zoologists, we are brought to realize that the original *S. plicatilis* described by Ehrenberg in 1838 is an entirely distinct organism and bears little relation to the other organisms which we now call "spirochætes." We also know that the latter should no longer be designated as spirochætes, and that the spiral organisms found in the crystalline style of various mussels are neither trypanosomes, as held by Perrin,[17] nor typical spirochætes, but form another group which may be seen to possess one or more genera. These facts were revealed after the death of Schaudinn by the careful studies of Novy and Knapp,[18] Schellack,[19] Gross,[20 21] Zuelzer,[22] Gonder,[23] Dobell,[24 25 26] Hoelling,[27 28] Fantham,[29 30] Swellengrebel,[31] Bosanquet,[32 33] and others. Although much light has been thrown upon the structure of these organisms, no definite conclusion has yet been reached as to the affinity of the "spirochætes" in the system of natural history. While there are still some who consider "spirochætes" as allied to bacteria and others who regard them as of a protozoan nature, there now appear to be certain authors who are inclined to set them apart both from bacteria and protozoa, and classify them apart in the domain of the Protista, i. e., belonging to neither plant nor animal. Dobell[24] represents this view, and Doflein[34] compromises by calling them "Proflagellate," and placing them between Bacteria and Protozoa. Zuelzer[22] holds a somewhat similar opinion to that of Dobell. In order to bring up some of the more important data relative to the question of classification, we shall now review the present situation.

As remarked at the beginning of this paper the Spirochæta of Ehrenberg was regarded as a genus of the family of Spirillacea, and no question was raised in regard to its possible affinity with the Protozoa until the publication of Schaudinn's fascinating observations on *Leucocytozoon ziemanni*. Since that time there have appeared numerous partisans of Schaudinn's view that so-called spirochætes are of protozoan nature. Their main contentions are as follows: (1) Longitudinal division as the mode of multiplication, (2) presence of an undulating membrane; (3) high degree of bodily flexibility; (4) absence of cell membrane; (5) absence of a motor organ such as the flagella; (6) presence of a periplastic process; (7) peculiar nuclear arrangement; (8) band-like bodies; (9) encystation or resistant form; (10) a certain periodicity in their pathogenic activity in the infected hosts, and (11) effect of certain chemicals such as sodium taurocholate, saponin, etc., which bring about the dissolution of these spiral organisms and thus offer a contrast to the great resistance shown by bacteria (especially spirillum) to these substances. The foregoing characteristics tended to place the Spirochæte in the Flagellate group, but subsequent studies by different investigators, especially those who have employed a more recent and approved cytological technic seem to indicate that many of the above criteria were based upon erroneous or insufficient observations. According to the observations of Dobell,[24] Gross,[16, 20, 21] Zuelzer,[22] Swellengrebel,[31] Novy and Knapp,[18] and others, the following features are characteristic of "spirochetes."

1. In the case of the majority of "Spirochetes" transverse division is the only mode of multiplication (Koch, Levaditi, Fraenkel, Novy and Knapp, Bor-

rel, Gross, Zuelzer, Swellengrebel, Schellack, etc.). Only in certain pathogenic small varieties has the occurrence of longitudinal division been reported.[7, 35]

2. No undulating membrane has been definitely demonstrated in any Spirochæta. The alleged undulating membrane depicted by Perrin[17] and Schaudinn[36] in the dried preparations of certain mussel spirochætes is an artefact brought about by improper fixation; namely, the torn crista of a cristispira.[20]

3. The alleged chromatin rods and spirals described by Perrin in the case of certain mussel spirochetes known as Spirochæta balbianii (Cristispira of Gross) are now said to be nothing but a distorted arrangement of volutin substance or chromidial granules which under optimum fixation gather themselves along the walls of the chambered structure of the cell body.

4. The absence or presence of cell membrane seems to depend upon the variety of "spirochætes." Thus the original type organism of Ehrenberg was described as being devoid of a membrane and is still so regarded by all who have studied this organism. On the other hand the mussel spirochætes and various small parasitic species are now said to be provided with a thin but elastic membrane which cannot be differentiated from the cell body by means of staining reactions. The presence of a membrane would suggest a close affinity with Spirillum, but the latter has a stiff nonelastic membrane.[22]

5. In regard to the motor organ no generalization can be made. The original type organism of Spirochæta and all mussed "spirochætes" are devoid of any special motor apparatus. On the other hand, a terminal process, consisting of a delicate, elastic filament with minute, regularly set curves, may in the case of various small parasitic spirochetes be found to project from one or both ends of the body. Borrel[37] and Zettnow[38] obtained some preparations in which Spirochætes of fowl spirillosis and relapsing fever appeared to possess peritrichal flagella, but this must have been a case of artefact formation, as no one has since been able to confirm their findings. Schaudinn considered the terminal process to be identical with the periplastic appendage of a flagellate.

6. Certain spirochætes such as *S. balanitidis* and *S. buccalis*, etc., were said by Schaudinn[36] Hoffmann and Prowazek,[39] to have a flattened, ribbon-formed body. Later investigators hold that the body is cylindrical and round on section.

7. Encystment, or the resting stage, such as observed in protozoan organisms, has been suggested[17, 40] as existing, but never satisfactorily proved.

It will be seen that the findings of later investigators deduct much of the foundation upon which the protozoan theory of "spirochætes" had been based. Not only do they separate the spirochætes from the Protozoa, but they also bring out certain new facts which make it difficult to include them among the Bacteria as was formerly done by those who opposed the view of their protozoan nature. As has been briefly remarked, the spiral organisms called spirochætes are not of uniform structure, but according to recent investigations, fall under several great divisions. It was owing to the imperfection of the methods of study that the free living forms and numerous parasitic varieties were at one time all held to belong to the same genus. Since the introduction of darkfield microscopy,[41] many points which could not be satisfactorily determined with stained specimens have been carefully checked up, and the entrance into the field of certain excellent

cytologists has helped to clear up many points relating to the systematic grouping of these organisms. These cytologists made extensive series of comparative studies, at the same time carefully examining the structure of bacteria, spirilla, spirulina, and oscillaria.

As has been pointed out by Bütschli,[42, 43] bacteria are composed of a central body and a plasmatic layer. The former contains volutin granules and some chromidial elements. The spirillum has a series of chambers, each of which is constructed like a single bacterial cell. Both are covered with a stiff cell membrane. The structure of Spirulina is similar to that of Spirillum, differing from the latter by the highly flexible character of the membrane. Now, a very similar structure was demonstrated by Gross[20] in the body of mussel spirochætes and speedily confirmed by Dobell,[24] Zuelzer[22] and others. Gross, Dobell, and Zuelzer all agree that the original free-living Spirochæta described by Ehrenberg is a unicellular organism which bears no relation either to the mussel spirochætes or to the small parasitic varieties. This fact implies the dissociation of the long used term Spirochæta from those organisms which in reality were commonly known as "spirochætes." Odd as it may seem, the true Spirochæta has been but rarely studied, even by biologists, and certainly not to any great extent by medical men who have so much to do with the so-called "spirochætes."

Gross[16] was the first person who proposed to distinguish the true Spirochæta from the other varieties of spirochætes by creating new genera for the latter which, according to his studies, could not be classified with Spirochæta in the strict sense of the term. Thus for the latter type he created the name Cristispira (those with Crista), for the large parasitic "spirochaetes" in fresh shell fish Saprospira (those without Crista), and the small parasitic varieties, including all pathogenic species, he designated as Spironema. Gross maintains that Cristispira, Saprospira and Spironema belong to the bacteria, and places them under the family name of Spironemacea. Gross[44] and Bosanquet[32] recorded a few instances in which certain mussel "spirochætes" went into spore-formation comparable to the true bacterial feature.

Dobell and Zuelzer both admit the striking resemblance between the chambered structure of Spirillum and Cristispira, but cautiously avoid accepting the bacterial theory of Gross on the ground that the last named organisms have a more elastic and flexible membrane and that they are not necessarily bacteria. Dobell, as has been stated, has proposed a new family name Spirochætoidea which should include not only Gross' Spironemacea but also Spirochæta. Dobell does not accept Gross' Spironema, as it was applied to a flagellate in 1892 (Klebs), but retains Schaudinn's Treponema to designate all small parasitic and pathogenic varieties. He does not consider that there is a sufficiently essential difference between them to warrant two genera. Zuelzer regards the affinity between the mussel "spirochætes" and Spirulina (one of the Cyanophycean genera) as being much closer than that between these types and Spirillum. On the other hand Gonder[45] accepts the classification of Gross more completely. He does not, however, share Gross' view that these organisms are definitely of a plant nature, holding that certain features indicate their partial affinity to the protozoa. He also differs from Gross in including Spirochæta under Spironemacea and in retaining

Schaudinn's term Treponema for the organisms of syphilis and yaws and such affections, while he accepts Gross' term Spironema for other varieties such as the "spirochætes" of relapsing fevers, tick fever, etc. The situation is still confused.

CLASSIFICATION AFTER GONDER (1912).

SPIRONEMACEA (GROSS, 1910).

Spirochæta (Ehrenberg, 1838)	Type: Spirochæta plicatilis, etc., all free living.
Cristispira (Gross, 1910)	Type: Cristispira balbianii and other varieties found in mussels.
Spironema (Vuillemin, 1905)	Type: Spironema recurrentis, and other parasitic and pathogenic varieties living in blood.
Treponema (Schaudinn, 1905)	Type: Treponema pallidum, Treponema pertenue, and other varieties with closely set spirals.

CLASSIFICATION AFTER GROSS (1912).

Spirochæta (Ehrenberg)	Type: Spirochæta plicatilis. Unicellular organism without a membrane or flagellum, highly flexible. Free living. Transverse division.

SPIRONEMACEA (GROSS, 1912).

Cristispira (Gross, 1910-11)	Including different varieties living in certain mussels. C. balbianii, C. anodontæ, C. pectinis, etc. All possess a crista. Chambered structure of the body. Sporulation. Transverse division.
Saprospira (Gross, 1911)	Similar to the foregoing except that there is no crista. Found in foraminiferous sand. Sporulation. Transverse division.
Spironema (Vuillemin, 1905)	Including small parasitic varieties: S. pallidum, S. pertenue, S. recurrentis, S. gallinarum, etc. Probably multicellular (or chambered). Transverse division. Flagella or terminal thread present.

CLASSIFICATION AFTER DOBELL.

SPIROCHÆTOIDEA (DOBELL) 1910-1911.

Spirochæta (Ehrenberg, 1838)	Free living forms, fresh water or marine. Spirochæta plicatilis (Ehrenberg) S. gigantea.
Treponema (Schaudinn, 1905)	Parasitic in animals, vertebrates and invertebrates. T. pallidum (Schaudinn), T. recurrentis, T. dentium, etc.
Cristispira (Gross, 1910)	Parasite in Lamellibranchiata (mussels). C. balbianii certes, C. anodontæ, C. pectinis, C. veneris.

CLASSIFICATION AFTER MIGULA (1897).

Bacteria	Coccaceæ, Bacteriaceæ, Spirillaceæ, Chlamydobacteriaceæ and Beggiatoaceæ.

SPIRILLACEÆ.

Spirosoma	Rigid; no organ of motion.
Microspira	Rigid; one seldom two or three, polar wavy flagella.
Spirillum	Rigid; polar tufts of 5-20 flagella, mostly semi-circular or wavy.
Spirochæta	Flexuous, motion organ unknown, probably an undulating membrane.

CLASSIFICATION AFTER SWELLENGREBEL.

- Bacteria....
  - Spirochætaceæ..... — More or less distinct properties of protozoa but not much more so than the bacteria capable of forming S or Fe in contrast to those producing nitrification. Plasmolysable like the Spirilla.
  - Spirillaceæ
  - Coccaceæ

CLASSIFICATION AFTER LEVADITI (1912).

SPIRILLACEÆ (MIGULA).

1. Sub-family, Spirillacea (Swellengrebel). Not flexible.
   - 1. Genus, Spirillum.
   - 2. Genus, Vibrio.

2. Sub-family, Spirochætacea. Spirally flexible, surrounded by periplastic cover, with or without flagellum-like appendage.
   - 1. Genus, Spirochæta. No preformed spiral; loss of spiral form at rest.
     - a. No flagellum with undulating membrane.
     - b. Flagellum, but no undulating membrane.
       - One or two terminal flagella.
         - S. buccalis.
         - S. balanitidis.
         - S. refringens.
       - Peritrichal flagella.
         - S. gallinarum.
         - S. duttoni.
         - S. obermeieri (?)
   - 2. Genus, Treponema. Preformed spiral; no visible undulating membrane.
     - a. Flagellum at one end.
       - T. pallidum.
       - T. framboesia
     - b. Flagellum at each end.
       - S. dentium.

Let us consider each of the groups in some detail on the basis of a newer classification.

Spirochæta.—According to Schaudinn,[36] the type organism of Spirochæta possesses certain features which are also found in trypanosomes—such as an undulating membrane, periplastic fibrillar process, longitudinal division, etc. But this apparent resemblance has been shown to be erroneous. Thus, according to the latest contributions made by Zuelzer,[22] the original type organism, *Spirochæta plicatilis,* has no chambered structure but is provided with a straight fibrillar axial filament surrounded by a plasmatic spiral layer which covers it unequally in different places. The organism consists of a single cell. Volutin granules which can be demonstrated by certain microchemical reactions are regularly disposed within the plasmatic layer. During motion the plasmatic layer

at a given position becomes thickened or reduced in volume according to the current of the substance. The spirals of the plasmatic layer surrounding the straight axial filament occur regularly and closely, while the whole body shows several irregular undulations. There is no flagellum or periplastic terminal process, and no membrane has been demonstrated. It measures 100-200 μ on an average, sometimes attaining a length of 500 μ, whereas it is only 0.5-0.75 μ in width. Unlike the other spiral organisms bearing the name of Spirochæta (undoubtedly indiscriminately applied) the members of this group of real spirochætes do not swim, but their locomotion is effected by a creeping movement along the surface of a supporting object. Multiplication is brought about by transverse division which is effected by a thickening of a certain part of the axial filament where a cross fissure takes place, followed by the strangulation of the plasmatic layer at the corresponding spot. Since Ehrenberg described the first species, four more species have been added, one by Cantacuzène[21] in 1910, and three by Zuelzer[22] in 1912. They are all free-living and are not known to be responsible for any pathological conditions in either human beings or animals.

Since the essential characteristics of the group of true Spirochæta do not agree with those of various other species hitherto unreservedly called spirochætes, the necessity of reclassification became apparent as soon as these facts were known about 1910, whereupon Gross, Dobell, and others undertook special studies in this connection. As has been mentioned, Gross, Dobell, and Gonder all possess their individual ways of classification, but all agree on one point; i. e., that the majority of the organisms known as spirochætes are not spirochætes in the strict systematic sense and must, therefore, be differently designated. Gross was the first to do this, and he was followed by Dobell and Gonder who introduced some modifications, but it seems that the family term Spironemacea of Gross has found a wider acceptance than Dobell's Spirochætoidea, although both include practically the same constituent organisms under a slightly different generic name. Thus Dobell accepts Gross' generic names Cristispira and Saprospira (provided that this genus can be recognized by other investigators) to cover the varieties found in shell fish, while preferring to use Treponema instead of Spironema as proposed by Gross. Dobell's family Spirochætoidea comprehends, besides all the constituents of Gross' family Spironemacea, the genus of the true Spirochæta. Whether the segregation of Spirochæta from the other genera composing Spironemacea is justified or not seems still debatable, inasmuch as the differences between the genus Cristispira and the genus Spironema are, I believe, no less striking than those which induced Gross to separate the Spirochæta from them. According to personal observations on small "Spirochætes" there seem to exist more affinities in the structure of true Spirochæta and the small parasitic varieties than are assumed by Gross and other investigators. For the present I will dwell upon different groups of organisms, which those investigators have classified as separate genera, and in order to give a basis for further development of the subject, I propose to employ the new generic names proposed by Gross, without, at the same time, committing myself to his views.

Cristispira.—This genus was created by Gross in 1910 for the large saprophytic commensal spiral organisms found in the alimentary canal of certain varie-

ties of shell fish. They are chiefly found in the crystalline style which is a jelly-like projection in the stomach. The most unique feature of the genus is the presence of a crista or ridge, which extends spirally along the whole length of the body, whence the name Cristispira. Certes[47] considered the type organism of the genus *Cristispira balbianii* to be a trypanosome on account of the presence of an undulating membrane (later recognized by Gross as a ridge) and it has been called Trypanosoma or Spirochæta indifferently. Laveran and Mesnil[48] in 1901 regarded it as allied to the bacteria. Perrin[17] in 1905-1906 took up the subject and arrived at the conclusion that it has many features in common with the trypanosomes. This he observed from stained preparations in which he found an undulating membrane, a spirally arranged nuclear rod, as well as various mitotic figures and longitudinal division. Perrin's observations were in part confirmed by Keysselitz,[49, 50] Swellengrebel,[31] Hoelling,[27, 28] Gonder,[23] and Fantham,[29] but a later investigation of Schellack[19] brought out an entirely different set of facts. According to Schellack the undulating membrane and spiral nuclear rod or alleged karyokinetic figures are an artefact caused by improper fixation (dry method). In properly fixed preparations the cell body is composed of an alveolar protoplasm and contains a number of transverse walls. In his later works Gonder[45] confirmed Schellack's observations. Zuelzer[22] and Dobell[24] found chromatin (and volutin) granules to be deposited along the surface of the transverse septa, while Gross[20] failed to see any chromatic granules in Cristispira. On the other hand, Hoelling thinks that the entire cell body is saturated with diffuse chromatin substance. The chambered structure of the cell body is regarded by Gross as a sign of the multicellular nature of the organism, but many authors hesitate to accept this view, maintaining that it is a single organism with numerous cross septa. Gross, Zuelzer, and Dobell all agree that the cell body is surrounded by a strong membrane similar to that found in bacteria, although Zuelzer distinguishes it from the latter by its high flexibility. They found that the membrane had a double contour and protected the cell body from the solvent action of various substances such as saponin as well as from acids and alkalies, a fact explained by Gonder as not necessarily due to the presence of a membrane but to the more concentrated external fibrillar layer on the cell surface. In fact, Gonder described a fibrillar appearance of the external layer of the cell body after the organism had been acted upon for some time by certain chemicals.[45]

Opinions still vary as to the origin of the ridge or crista. Earlier workers viewed it as an undulating membrane.[17] Gross, Zuelzer, and Dobell hold that it is a superposed structure having no direct connection with the cell body, while Schellack regards it as a true periplast traversed by numerous fibrils. He believes that the so-called undulating membrane of the authors of the Cristispira is an artefact produced by defective technic. Hoelling, as well as Fantham and Porter, entertains a view similar to that of Schellack, and the presence of a myoneme in the periplast was even maintained by Fantham and Porter. Mackinnon[51] and Vlès[52] were unable to demonstrate any myoneme in the periplast, although Borrel and Cernovodeanu[53] assume that there exists a myoneme in the membrane which enables it to flatten or fold the ridge. When the organism is subjected to macerating or solvent agents (saponin, acid, alkali, etc.) the mem-

brane is first attacked. The delicate fibrils become quite distinct in the course of dissolution, but the whole structure finally disappears completely, showing the plasmatic nature of the membrane. The cell body is much more resistant.

Division is exclusively transverse, according to the investigations of Schellack, Gross, Zuelzer, Dobell, Laveran, and Mesnil, while earlier investigators (Perrin, Keysselitz, Gonder, etc.) considered it longitudinal. Fantham and Porter,[35] working with *S. obermeieri* and *S. duttoni*, found both modes of division occurring. It is possible that a peculiar mode of division, described by Gross[20, 34] as an *incurvation*, might have been the cause of mistaking it for longitudinal division. Incurvation is a phase of the transverse division of Cristispira, whose body first doubles up (incurvates) at the segment where the fission is to take place and then after some time completes the process. During the incurvation both halves of the organism intertwine and simulate a stage of longitudinal division.

Sporulation was described by Gross[44] who saw a Cristispira produce a series of somewhat smaller, highly refractile, oval bodies out of the square chambered structure of the cell body. These oval bodies were seen to separate into individuals, but no new cristispira could be made to sprout out of these bodies (or so-called spores). Bosanquet[32] made a similar observation. The question of sporulation is still open to further confirmation and is very important in view of the divided opinion regarding the affinity of this group in the system.

The cell body is highly flexible, round on section, wavy or spirally wound, possessing not more than three or four curves. There are neither flagella nor terminal projections, except in one small species, *C. spiculifera*, which Shellack described as having a terminal filament.

There are about 18 known species which inhabit different varieties of shell fish belonging to nearly twelve different genera of Lamellibranchs, including common oysters and fresh water mussels. These genera are Ostrea, Anodonta, Chama, Pinna, Mactra, Pecten, Modiola, Lima, Gastrochæna, Saxicava, Tapes, and Umo. *Cristispira balbianii* and *C. anodontæ* are the largest species and measure 100-130 μ in length and 3-5 μ in width, while the smallest representative of the genus, *C. papillosum*, measures but 18.5-20 μ by 1.1-1.4 μ.

Saprospira.—Gross[20] proposed to introduce this genus in order to group together a new species of mussel "spirochætes" which distinguished themselves from Cristispira by the absence of a crista. Their habitat and other cytological features are the same as those noted in the Cristispiræ. According to this investigator, *Saprospira grandis* and *S. nana* undergo multiple transverse division and bear a more distinctly bacterial aspect.

Spironema and Treponema.—Under Spironema, Gross classified all the pathogenic and small saprophytic varieties. Dobell[24] substituted Spironema for Schaudinn's Treponema on the basis that the former term was applied to a flagellate, *Spironema multiciliatum*[15] by Klebs in 1892; for he did not consider it necessary to create two genera out of these organisms. Gonder still hesitates to drop the distinction between the group of "blood spirochætes" and that of "tissue spirochætes," the latter containing *Treponema pallidum* as type organism. While Schaudinn's original criteria for Treponema are no longer valid as regards several

points, Gonder proposes to retain the term Treponema for the pallidum group and to accept Spironema for the more irregularly curved, wavy varieties to which most of the "blood spirochætes" and saprophytic parasites belong. From personal observations I believe the differences between the two groups to be differences of degree, not of quality. They should belong to one and the same genus as may be seen from the characteristics enumerated below. Spironema and Treponema have a slender, cylindrical, spirally wound, highly flexible body, which exhibits serpentine, cork-screw-like, and sometimes lashing movements. The spiral curves are partially stretched and drawn together with a certain rhythm, so that an actively motile organism resembles a spiral spring which is alternately drawn out and relaxed. When reduced in motility the organism may rotate along its axis in one and then in another direction without changing its curves. In certain species a lateral bending or swinging motion of one half of the body may be seen. It seems to be the general rule that the more active and energetic an organism is, the less rigid are its curves. On the whole the pallidum group (Treponema) exhibits a less energetic motility than the heavier group (Spironema) which it relinquishes much sooner than the latter. Therefore, it is only in perfectly fresh material (such as that obtained from an experimental syphilitic lesion in animals at the moment of examination) that the stretching of the curves, as in the case of so-called Spironema, can be recognized. This point can be clearly demonstrated in a section of syphiloma in a rabbit's testicle fixed immediately after removal from the animal. Here we find the organisms showing most striking irregularity of curves very unlike the accustomed picture of regularly curved specimens found in a section obtained from postmortem material, such as a tissue from macerated congenitally syphilitic fetus (Flexner)[55] or from a preparation made after the organism has become sluggish. The reverse is also true. A spironema from a case of relapsing fever is always wavy and irregularly curved in a stained preparation, but it is much more regular when observed under the dark-field microscope and becomes completely regular when nearing death as a result of being exposed to progressively unfavorable conditions. In a culture where the motility is somewhat less active the organism appears just as regularly curved as a treponema. The sudden death of these organisms leaves them in a state of motion, hence their irregular curves.

The body of Spironema is much heavier than that of Treponema and in relation to different dyes it may be stated that the former takes on a more bluish component of Giemsa's solution than the latter, which usually takes on the red. In regard to the structure of the cell body, the minuteness of these organisms precludes the possibility of obtaining much information by means of our present methods of differentiation. Many authors assume the presence of a membrane analogous to the periplast of a flagellate and believe that it can be demonstrated by means of maceration. In one species of Spironema, Prowazek[40] assumed a central axial filament surrounded by a layer of cytoplasm. The active motility exhibited by these organisms led some investigators to suggest the existence of contractile fibrils or a myoneme in the cell body. My observations on fresh specimens obtained from pure cultures of these organisms support the view that these *spirochætes* are provided with an axial spiral filament covered with a layer of

protoplasm. On the surface of the cell body there is a thin membrane which can be detected when the organism undergoes degeneration. At this stage the cytoplasm becomes so rarefied—i. e., it escapes from the space which it occupied—that the axial filament and the membrane can be easily recognized. In a subsequent phase the membrane also disappears, leaving the axial filament denuded. This is a common phenomenon in the cultivation of this group of organisms. Schellack[19] maintains that the external layer of the cell body stains red with iron hematoxylin eosin, while the inner layer takes on a dark bluish tint, hence the former is of ectoplasmatic and the latter of endoplasmatic origin. Gonder[56] describes an ectoplasmatic layer in *Spironema vesperuginis*. Fantham and Porter[35] as well as Prowazek[40] mention the existence in Spironemata of an undulating membrane, as was originally suggested by Schaudinn[36] owing to a wavy movement which he observed to travel through the body of a resting spironema. Gross and Zuelzer failed to demonstrate any such particular structure. Another important feature of Spironema and Treponema is the presence of a terminal appendage projecting from the end of the cell body. The bodies of Spironemata and Treponemata taper at both extremities from which is sent out a very fine terminal thread, at one or both ends. The length of the terminal appendage may reach ⅓ to ½ of the body and is immeasurably thin. In old cultures, especially when grown in a fluid medium, these terminal appendages are much heavier and more easily recognized than in a specimen derived direct from the natural habitat. The terminal filament is provided throughout its length with numerous, closely set, regular curves.[57] It is rigidly joined at the pointed ends of the body or sometimes in such a loose manner as to permit the joint to bend at any angle to the long axis of the organism. No proper motility can be discerned in the appendage, which is elastic. In certain specimens an active swinging or jerking movement can be seen to be transmitted by the organism, which is able to do this by means of its contractile element (myoneme?) contained within the body. In several instances in which the cultivated *Spironema recurrentis* had been exposed to the solvent action of certain chemicals (saponin, sodium taurocholate, etc.), I have observed many denuded axial filaments (their cytoplasmic layer having been dissolved) to which the terminal filaments were also attached. Suddenly I saw some of the terminal projections commence active jerking and swinging motions. The skeletal axial filaments still remained. By means of careful examination it was found that there was a pair of highly refractile, round bodies attached to the skeletal filaments near both extremities. These bodies, which measured about 0.5 μ in diameter, appeared to have some contractility as suggested by the alternate change in the degree of the refraction of light. Whether or not these bodies represent some sort of myonemous elements cannot be definitely stated, but it is significant that similar nodules, if not in pairs, can be seen to travel from one point to another in an actively motile spironema. Prowazek[58] once called attention to the phenomenon of plasmatic condensation in the body of *Spironema gallinarum*.

The nature of the terminal appendage is not known. Many authors (Hoffmann, Prowazek, etc., on *S. buccalis* and *S. balanitidis;* Novy and Knapp on *S. recurrentis*) view it as a prolongation of the periplastic fibrils which are in

connection with the periplast. Others regard it simply as a drawn out part of the cytoplasm produced at the line of division. I am inclined to think that the terminal projection with regularly set curves is a separate part not directly connected with the membrane, nor existing as a prolongation of the axial filament. It is connected with the cell extremity by means of a tendinous substance. It resembles the flagellum of certain bacteria, inasmuch as it is similarly elastic, finely set with regular curves, and visible under the darkfield microscope. On the other hand, a great many of the bacterial flagella cannot be demonstrated in a fresh preparation even by means of a darkfield illumination. Zettnow,[38] Borrel[37] and Fraenkel[59] obtained preparations of *S. recurrentis, S. gallinarum* and *S. duttoni* in which peritrichal "flagella" were shown by means of flagella staining methods, but these flagella-like fibrils are now regarded as fibrils which have become detached from the external layer of the organisms through maceration. By means of the lucidol method of Szécsi,[60] Gonder[45] succeeded in staining one fine terminal projection at each end of *S. recurrentis* as did also Wolbach by the adoption of Casares Gil's[61] method.

There are several views regarding the mode of multiplication. The theory most generally accepted is that these spironemata undergo transverse division like bacteria, differing from the latter, however, in not forming a wall at the point of division. The division is effected by means of a thinning out process of the protoplasma which for a time bridges the two newly formed daughter cells. Finally they separate by the severance of the connecting thread. Novy and Knapp[18] described a cleft formation at the point of division. The view of the transverse division is held by Koch, Novy and Knapp, Metchnikoff, C. Fraenkel, Borrel, Laveran, Sobernheim, Gross, Thesing, Schellack, Nakano,[62] and others. On the other hand Schaudinn, Hoffmann, Hartmann, Keysselitz, Herxheimer, Prowazek, Gonder, Fantham, and Porter support the theory of a longitudinal division as in the flagellates. Indeed, Krysztalowicz and Siedlecki[63] in 1905 went so far as to propose the term "Spiroflagellata" under Mastigophora. I have also observed instances in which the phenomena could only be explained by longitudinal division. Thus, in pure cultures of various spironemata and treponemata we find forms in which a longitudinal cleft can be traced in the somewhat heavier specimens. The cleft may run but a short distance, or one-third, one-half or almost the entire length of the body. In some specimens the cleft widens up and causes one-half of the body to be split into two limbs (two daughter cells in half separation). Observed under the darkfield microscope the process is seen to be slow. It may be added that it is tedious to actually follow up the entire process of any mode of division under the microscope, no matter whether this be transverse or longitudinal. As may easily be conceived, those who hold the theory of transverse division argue that the forms held by their opponents to be a stage of longitudinal division are formed by two entwined spironemata which having been produced by transverse division are still connected by a delicate plasmatic bridge. This argument, however, can also be used in the reverse sense in favor of longitudinal division, as it is also possible that the two daughter cells which have just undergone cell division can remain united at their ends, thus bearing the appearance of representing a stage of transverse division. A strong support in favor of the

transverse mode of multiplication lies in the formation of a very long thread consisting of several sections united together by means of a delicate bridge between them. This phenomenon is of common occurrence in any spironema or treponema culture. It is highly probable that the usual mode of division in culture is transverse, although the possibility of longitudinal division cannot be excluded. Recently Meirowsky[64] advanced the view that Spironema and Treponema besides multiplying transversely also do so by a process of fructification (Doldenbildung) and budding (Knospbildung) similar to that observed in some lower plant organisms. His ideas were chiefly based upon phenomena observed by means of various methods of vital staining, in a culture of *Treponema pallidum* (furnished by Sowade). He describes numerous granules collected in a group at one point or another along the body of the pallidum and also branching out of sprouts from some of the specimens. There are many factors to be taken into consideration in such an experimental arrangement which will make it difficult to properly estimate the value of the observations. Those made under the microscope on a preparation containing the organisms, consisting of semi-coagulated horse serum, solution of precipitable aniline dyes (effected particularly through a change of reaction in the medium) are of a disputable character when we consider the absence of strict aseptic precautions as well as the comparatively long period of observation (many days and weeks) during which a preparation had been kept for observation. It is possible that under these unfavorable conditions various forms of involution result which do not appear under normal cultural conditions. Certainly it is not convincing that this so-called fructification or budding also occurs in the body of infected hosts.

Balfour[65, 66] noticed the appearance of certain granules within some of the erythrocytes of fowls which had just stood the first attack of the Sudanese fowl spironematosis and thought that these granules give rise to a new generation of the spiral forms of the organism which reappear at the second attack. That is to say, that a Spironema found by Balfour in a Sudan epizootia possesses a spiral and a granular phase of life. Leishman,[67] Blanc,[68] Fantham,[69] Nuttall,[69a] and Hindle[70] also entertain the belief that *Spironema duttoni* and *Spironema gallinarum* adopt a granular form under certain conditions, and that a spiral form can sprout out when the conditions become favorable. Thus in the body of infected ticks these spiral organisms undergo segmentation and numerous granules are produced, a process analogous to sporulation. These granules were called by the authors coccoid bodies, infective granules, or spores. This view was supported by the histological studies of Hindle who secured a series of preparations in which these granules can be demonstrated in the body of the tick. According to Hindle these granules become spiral when the infected tick is incubated at 37° C. for a certain time. In contradistinction to the above findings, Marchoux and Couvy,[71] Gleitsmann,[72] Gonder,[45] and Todd and Wolbach[73] maintain that in an infected tick some motile spironemata can always be demonstrated and that the granules described by Hindle and others are not specific for the infected ticks, but can also be found in the control specimens. Fantham[69] points out, however, that the granules of normal ticks are not identical with the coccoid bodies of Spironema found in the infected ticks.

Schaudinn, Prowazek, and others noticed that certain species formed nodules under adverse conditions and suggested that these may represent a resting stage (or resistant form); but Schellack and Wolbach regard them as a depression phenomenon which can also be induced by prolonged treatment of the organisms with a saline solution. Besides, there is a peculiar, highly refractile, round body which is very often found attached somewhere along the side of the body of the organism. There may be one or more such bodies in a specimen. The significance of this body is still obscure, but it may possibly be caused through a disturbance of the osmotic equivalence existing between the cytoplasm of the organism and the medium, not unlike the phenomenon known as plasmoptysis. I have demonstrated its occurrence in the cultivated specimens of various species of Spironema and Treponema. The body is more frequently present in an old culture in which innumerable granules are also found. In certain culture tubes these minute granules are mostly of varying size. By making a transplant of such a culture into a new medium it was found that, when examined several days later, the new culture contained many short spiral forms which were in one manner or another intimately connected with the granules. This phenomenon suggested the possibility of representing the sprouting of the spiral forms from the granules.

PATHOGENICITY.

Spironemata and treponemata are parasitic, and some varieties are responsible for various diseases in man and animals. Various forms of acute febrile diseases, as well as chronic pathological conditions are caused by the invasion of the blood or tissues by this group of organisms. It may be mentioned that the spironemata are almost always transmitted from a sick individual to a normal person through the intermediary of certain blood-sucking insects and invade the blood principally, whereas the pathogenic treponemata are carried from man to man by direct contact and show a predilection for various organs and tissues. As a rule the phase of the spironemal infection is acute and brief and that of the treponemal invasion runs a chronic course, as instanced in the former case by the type of relapsing and tick fevers and in the latter by syphilis and yaws.

Besides the pathogenic species there are a large number of saprophytic varieties belonging to these two genera (or one, according to certain classifications) which are common inhabitants of the oral cavity, genitalia, and alimentary tract of man and animals. Some forms are frequently associated with certain pathological conditions, but their etiologic significance has not been definitely determined. Such is the case with *S. balanitidis* in Ulcus erosina circinata, *S. vincenti* in an acute angina, *S. schaudinni* in Ulcus tropicus and *Treponema mucosum* in pyorrhea alveolaris, etc. It may be that some of these play the rôle of a secondary invader and aggravate the conditions.

In the following table, I have enumerated the different species of Spironema and Treponema which have hitherto been observed by various investigators throughout the animal kingdom. It will be seen that the search has been more thorough in the case of the warm-blooded vertebrates than the cold-blooded orders, while even mosquitoes, ants, mites, and fleas are found to harbor certain species of these organisms.

SPIROCHÆTÆ (LARGE FREE-LIVING FORMS).

Sp. plicatilis.* Fresh water. 100–200μ, 500μ max.×0.5–0.75μ Ehrenberg, 1838.
Sp. plicatilis marina*.....Zuelzer, 1912.
Sp. plicatilis eurystrepta*.....Zuelzer, 1912.
Sp. stenostrepta*.....Zuelzer, 1912.
Sp. daxensis†.....Hotspring. 30-100μ×0.5-0.75μ Cantacuzene, 1910.

*Zuelzer cultivated these varieties in a suitable medium and proved each of them to be different from the others; hence she made subspecies. Schaudinn considered them to represent male and female forms.

†In the water of hot springs of Dax (52°-56° C.).

CRISTISPIRÆ AND SAPROSPIRÆ (LARGE SAPROPHYTIC AND COMMENSAL FORMS IN THE ALIMENTARY CANALS OF SHELLFISH).

Cristispira balbianii*....Ostrea angulata
O. edulis.....100–120μ×3–5μ. Certes, 1882.
C. anodontæ.....Fresh water mussel,
A. cygnea; also
A. mutabilis. 130μ×3–4μ.....Keysselitz, 1906.
C. spiculifera†.....ditto.....28–36μ×0.7–1.1μ. Schellack, 1909.

C. primæ††.....Prima squamosa,
P. nobilis.....10–60μ×0.5–3μ....Gonder, 1908.
C. mactræ.....Mactra sulcataria 45–70μ×0.8–1.0μ..Prowazek, 1910.
C. pectinis.....Pecten jacobæus..72μ ×1.5μ.....Gross, 1910.
C. interrogationis.....25μ ×0.5μ.....Gross, 1910.
C. veneris.....Venus casta.....Dobell, 1910.
Saprospira grandis.....Gross, 1912.
S. nana.....Gross, 1912.

*Once considered to be trypanosome or spirochæta.

†Bosanquet doubts its being a separate species from C. anodontæ.

††Gonder once described a blepharoplast near one blunt end; nucleus in single rod or irregular masses. Specimens with rod-formed nucleus may be male elements, since they are highly active, the others female or indifferent elements. A concentration of all the chromatin into one rounded mass was sometimes observed. Encystment also occurs. Gonder no longer upholds his above-cited interpretations, explaining them on the ground of faulty technical handling of the preparation.

	(SCHELLACK)	Length Average.	Length Extremes.	Length Average.	Length Extremes.	Ends.
C. balbianii	Ostrea edulis	39	35-42	1.3	1.1-1.5	Rounded, no t. ap.
C. ostræ	Ostrea edulis	41.5	38-42.5	1.1	1.0-1.3	Sharp, no t. ap.
C. chamæ	Chama gryphoides / Ch. sinistrorsa	45.6	45-46.5	1.4	1.3-1.5	Rounded, no t. ap.
C. anodontæ	Anodonta mutabilis	46	39-50.5	1.0	0.9-1.2	Rounded, no t. ap.
C. spiculifera	Anodonta	33	28-36.5	0.9	0.7-1.1	Pointed, t. filam.
C. modiolæ	M. barbato	37.5	36-40	0.8	0.7-0.9	Rounded, no t. ap.
C. primæ	P. nobilis	30.4	29-31	1.0	0.8-1.1	Rounded, no t. ap.
C. limæ	L. inflato, L. hiano.	37	35-41	1.4	1.0-1.8	Rounded, no t. ap.
C. cardii papillose.	C. papillosum	19.1	18.5-20	1.2	1.1-1.4	Rounded, no t. ap.
C. tapetos	T. decussata	34.5	29-35	1.3	1.1-1.4	Rounded, occasional t. ap.
C. acuminata	Tapes læta	37	43.5-49.5	1.0	0.9-1.1	Pointed, no t. ap.
C. saxicavæ	Sax. arctica	31	30-32	1.7	1.6-1.8	Rounded, no t. ap.
C. gastrochænæ	G. dubia	29	constant	1.2	1.1-1.3	One end blunt, one sharp, no t. ap.
S. pusella*	Anodonta, Umo, Lima, Tapes, etc.	13	12-14	..	0.3-0.4	Sharp pointed.

*Bosanquet found a spirochæta 10-12μ in length which he thinks may be identical with Spirochæta hartmanni of Gonder or with S. pusella of Schellack. No crista?

SPIRONEMA.

S. obermeieri*	Man, Europe	8-16μ×0.25μ	Cohn, 1877.[74]
S. carteri	Man, India	8-16μ×0.2μ	Mackie, 1907.[75]
S. duttoni	Man, West Africa	16-30μ×0.2μ	Novy and Knapp, 1906, Breinl, 1906.[76]
S. kochi	Man, East Africa		Schellack, 1907.[77]
S. berbera	Man, Algiers	12μ	Sergent, 1908.[78]
S. ægyptica	Man, Egypt	13.5μ	
S. novyi	Man, North America	12μ	Schellack, 1907.[77]
S. icterohemorrhagiæ	Man, 4-9μ×0.3μ, exceptionally 25μ		Inada, 1914-15.[79]
S. nodosum	Man		Huebener and Reiter, 1916.[80]
S. gallinarum†	Fowl		Marchoux and Salimbeni, 1903.[81]
S. anserina	Goose		Sacharoff, 1890.[82]
S. theileri	Cattle,	20-30μ×0.25-0.33μ	Läveran, 1902.
S. bovis caffris	Cattle		Nuttall, 1910.
S. equi	Horse		Novy and Knapp.
S. equina	Horse		Theiler, 1906.[83]
S. ovina	Sheep		Blanchard, 1906.
S. macaci	Inacacus, Ceylon		Castellani and Chambers, 1908.
S. pitheci	Cereopethicus pates French Sudan		Thiroux and Dufongéré, 1910.
S. lutræ	Otter		Prowazek, 1907.
S. lovati	Grouse's coccum	16-35.5μ×	Fantham, 1910.
S. vesperuginis	Tunisian bat	12-18μ×0.25μ	Gonder, 1908.
S. lagopodis	Grouse's blood	10-18μ×	Fantham, 1910.
S. laverani	Mouse	1.8-3.75μ×0.1-0.2μ	Breinl and Kinghorn, 1906.[84]
S. suis	Pigskin lesion or tumor	6-12μ	Dodd, 1906, Cleland, 1906.
S. muris	Rat	3-7μ×0.2μ	Wenyon, 1906.[85]
S. minor	Rat	5-9μ	Carter, 1887.[86]
S. microgyratum	Ulcerated cancers,	5-11μ×1.5-2μ 2.5-6μ×0.16-0.25μ	Löwenthal, 1906.[87]
S. eugyratum	Human intestine, stenogyr.	4.6-7.3μ	Werner, 1906.
S. stenogyratum	Human intestine,	3.6-6.7μ	Werner, 1906.
S. gondii	Rodent Ctenodactylus gondi	16-19μ×0.3μ	Nicolle, 1907.
S. gadi S. W.	Fish, Gadus minutus	10-16μ×3.5-4μ	Neumann, 1909.
S. pelanchysis S. W.	Pelamys sarda	9-10μ×1-1.9μ	Neumann, 1909.
S. jonesii, F. W.	Fish, Clavias angolensin	18μ×0.1μ	Dutton, Todd and Toby, 1906.
S. hartmanni	Prima squamosa, P. nobilis intestine	6-14μ×1μ	Gonder, 1908.[88]
S. bufonis	Bufo vugaris Rectum	8-10μ×1.5μ	Dobell, 1908.
S. minei	Work. Ants. Termes lucifugus	15-50μ×0.3-1μ	Prowazek, 1910.
S. glossinæ	Tse-tsefly stomach	8-15μ	Novy and Knapp, 1906.
S. culicus	Gnat. aliment. canal large		Jaffé, 1907.
S. buccalis††		12-20μ×0.5-xμ	Cohn, 1877.
S. vincenti	Pharyngitis	10-40μ	Blanchard, 1906.[90]
S. gracilis	Abscess near jaw		Vesprémi, 1907.[91]
S. schandinni	Tropical ulcer		Prowazek, 1907.[92]
S. pseudopallidum	Various ulcers		Mulzer, 1905.[93]
S. bronchialis	Bronchitis in Ceylon	15-30μ	Castellani, 1907.[94]
S. phagedenis	Phagedenic ulcer in man		Noguchi, 1912.[95]
S. refringens		8-12μ×0.33μ	Schaudinn, 1905.[6]
S. balanitidis	Balanitis	8-12μ×0.5-0.75μ	Hoffmann and Prowazek, 1906.[39]
S. obtusum	Yaws lesion		Castellani, 1905.[96]

SPIRONEMA (CONT'D).

S. acuminatum	Yaws		Castellani, 1905.[96]
S. aboriginalis	Ulcerative granuloma on pedenda	18-20μ	Cleland, 1909.[97]
S. interrogans	Yellow fever	14μ×0.17μ	Stimson, 1909.
S. hyos	Hog cholera		King, Hoffmann, Bæslack, 1913.[98, 99, 100]
S. grassi	Termite in Italy		Doflein.
S. termitis	Termite in Ceylon large		Dobell, 1910.
S. ctenocephali	Dog flea		Patton.

*Synonymous with S. recurrentis, Lebert, 1874.

†There are three subspecies: S. granulosa penetrans, in Sudan; S. nicollei in Tunis, and S. neveuxi in Senegal.

††Subspecies: Undulata and inequalis.

Lingard described Spironema in the blood of the camel, dog, elephant and horse; James, in an ulcer of the dog's muzzle, and Lucet in the gastro enteritis; Mathias and Leger in the blood of the zebra and antelope; Bell and Ruquet in the stomach of a normal dog; Dobell in Tropidonotus stolatus; Mühlens and Gleitmann in the boa constrictor; Ed. and Ét. Sergent in the alimentary tract of anopheles maculipenis larva; Mühlens often found spirochætes resembling S. recurrentis in the Culex mosquitoes.

TREPONEMA.

T. pallidum	Syphilis	6-14μ×0.2-0.25μ	Schaudinn and Hoffmann, 1905.[6]
T. pertenue	Yaws		Castellani, 1905.[101]
T. microdentium		3-12μ×0.2-0.25μ	Noguchi, 1912.[102]
T. dentium‡			Koch, 1877.

‡Subspecies: S. recta, S. tenue, S. denticola.

T. macrodentium		6-18μ×0.3-0.5μ	Noguchi, 1912.[102]
T. mucosum	Pyorrhea alveolaris	3-12μ×0.2-0.25μ	Noguchi, 1912.[103]
T. calligyrum	Condyloma		Noguchi, 1912.[104]

The foregoing list may be classified according to the habitat of the organisms. Thus, when they multiply within the blood circulation of man or animals they may either lead to a grave pathological condition or may produce no appreciable disturbance of the host that harbors them. In the case of certain tissue-invading species, serious pathological consequences may ensue or the host may remain more or less indifferent to the parasite.

1. Varieties which invade the blood principally.

(A) *Those which cause characteristic fevers known as relapsing or tick fever (pathogenic).*—There are seven for man, five for mammals and, at least, two for birds. These are:

For man: Spironema obermeieri (in European relapsing fever), *S. carteri* (in East Indian relapsing fever), *S. duttoni* (African tick fever), *S. kochi* (in East African tick fever), *S. novyi* (in American relapsing fever), *S. aegyptica*, and *S. berbera* (in Egyptian and North African relapsing fever).

For mammals: Spironema theileri (in South African cattle fever), *S. equi* and *S. equina* (in horse), and *S. ovinae* (in sheep), *S. macaci* and *S. pitheci* (both in East Indian monkeys).

For birds: Spironema gallinarum (in South American and African chicken fevers), *S. anserina* (in goose fever).

(B) *Those which do not seem to produce any grave condition, but are incidentally found (nonpathogenic).*—

For man: None.

For mammals: Spironema lutrae (in otter), *S. gondii, S. vesperuginis* (Tunician bat), *S. muris, S. minor* (both in rats), *S. lavcrani* (in mouse). The organism found by Macneal[105] may be identical with *S. muris.*

For birds: Spironema lagopodis (in grouse's blood).

For reptiles: Spironemata found in Tropidonotus and Boa.

For fish: Spironema gadi, S. pelamydis, S. jonesi.

2. Varieties which invade the tissue principally.

(A) *Those which cause characteristic lesions and symptoms (pathogenic).*—In this group there are no Spironema, but the only two known varieties belong to Treponema. No pathogenic tissue parasite belonging to Spironemaceæ was found in animals. The two pathogenic treponemata for man are *Treponema pallidum* (in syphilis), and *T. pertenue* (in yaws).

(B) *Those which do not seem to cause any noticeable lesion.*—To this belongs a Spironema (or Treponema) discovered by Gaylord[106, 107] and Borrel[108] in mouse cancers. Similar organisms were found by Tyzzer,[109] Deetjen,[110] and Mezinescu.[111]

3. Varieties which invade both the blood and the tissues indifferently.

Spironema (?) icterohemorrhagiæ (in Weil's disease prevalent in Japan) and *S. nodosum* (in Weil's disease prevalent in Germany) are the only ones so far known to come under this heading. The former, first discovered by Inada, is probably identical with *S. nodosum* of Huebener and Reiter, who also found it independently of Inada a year later. Stokes confirmed the work of Inada on the cases prevalent in Flanders.*

4. Varieties which may be associated with certain pathological conditions and some of which are regarded as having a more intimate relation to the lesion than that of mere secondary invaders.

There are about seven Spironemata and one Treponema which have been recorded in man and may be included in this category. These are: *Spironema vincenti* (acute pharyngitis), *S. schaudinni* (in tropical ulcers), *S. bronchialis* (in pulmonary gangrene), *S. balanitidis* (Ulcus erosiva circinata), *S. gracilis* (ulcerating jaw), *S. aboriginalis* (ulcerative granuloma), *S. phagedenis* (in phagedenic ulcer). The only Treponema which may be constantly associated with pyorrhea aveolaris is *T. mucosum. S. suis* was found in the ulcerating lesions of pigs.

5. Varieties which are found in or about the body cavities, alimentary tract, and genitalia are nonpathogenic and numerous.

For man there are described eight species of Spironema and five species of Treponema. Some of them have been known for many years while others are of recent addition. It may here be noted that similar flora are encountered in mammalian animals. These are as follows:

Spironema refringens, S. microgyratum, S. buccalis, S. acuminatum, S. obtusum, S. pseudopallidum, S. eugyratum, S. stenogyratum, and *Treponema macrodentium, T. medium, T. microdentium, T. dentium, T. calligyrum.*

S. hyos discovered by King and Bæslack[98] in the blood of pigs suffering from hog cholera is considered by them to be the cause of this disease.[99, 100] This or-

*Personal communication from Dr. Adrian Stokes, Captain R. A. M. C.

ganism should be more extensively studied, particularly in its relation to various spirochætes found in certain conditions in this animal.[112, 113]

The Spironema flora in birds, reptiles, fish and amphibia is practically unexplored, but we find one *Spironema bufonis* in a toad.

For insects there are on record several spironemata; namely, *S. culcis* (culex mosquito), *S. termitus*, *S. grassi* (both in mites), *S. ctenocephali* (in dog fleas).

Before leaving this chapter it may be well to dwell somewhat more extensively on the two recently discovered pathogenic spironemata. Brief mention has been made of the one, namely Inada's *S. icterohemorrhagiæ*, causing Weil's disease. The other, discovered by Futaki, Takaki and Taniguchi[114] in the blood and glandular tissues in two cases of rat-bite fever in Japan, is a Spironema believed by them to be allied to *S. recurrentis*.

Since Weil[115] called attention to the existence of an infectious disease characterized by a sudden onset, chills, high fever, muscular pains, jaundice, occasional hemorrhages in the skin, and acute nephritis, there have appeared numerous contributions to establish the entity of the disease. It has been found to break out among the soldiers in a barrack or among butchers or sewage-drainers. The mortality in European epidemics was rather low (about 15 per cent). The disease reaches its maximum on the 9th or 10th day, and then gradually ends in a lysis which retards the recovery of health for about a month or longer. Death occurs before the end of the second week of the illness. A similar disease has been known in Japan for the past several years. It was reported in certain districts (Kiushiu, Chiba, Shikoku) to have claimed many thousands of victims every year among the farmers, miners, and other laborers. Children under ten years of age are seldom affected, while those who are more occupied in field work contract the disease more frequently. There seems to be no authentic instance in which the disease was carried to another individual by direct contact. While many bacilli and cocci had been isolated and temporarily held to be the causative agent of this disease, no conclusive evidence had been adduced to support them. Jaeger[116] once described a *B. proteus fluorescens* as the cause of Weil's disease, prevalent in 1892.

Since 1912 Inada and his associates[79] have undertaken an extensive experimental study of this disease, and in 1914 succeeded in transmitting it to guinea pigs. Macacus, rabbits, rats and mice were partially or completely refractory to the inoculation. The most important points of the work of these investigators are (1) the reproduction of the typical symptoms (fever, jaundice, acute nephritis, swelling, and fatty degeneration of the liver, generalized hemorrhages, subnormal temperature before death, etc.); (2) the fact that successful inoculation of the guinea pig depends upon the period of the disease at which the blood was drawn from the patient; namely, no positive result was obtained with material taken after the first week of illness; and (3) the discovery of the Spirochæta, *S. icterohemorrhagiæ* in the blood, visceral organs, glands, affected skin, and muscles, both in man and the experimentally infected guinea pig.

It must be mentioned that the discovery of the Spironema was first made early in 1915 with the tissues and blood of the guinea pig, as the organisms are more abundant in experimental Weil's disease than in human cases. In October, 1915, an opportunity was afforded me to observe a number of cases of

this disease occurring in Chiba and, through the cooperation of Dr. Miyajima, some material for experimental studies was collected. One of the patients had had the attack a month previously and was at the convalescent stage. He was anemic, thin, and moderately jaundiced. The urine (dark, turbid) was collected and inoculated into the peritoneal cavity of the guinea pig. The animal started to show the typical symptoms (fever, jaundice, epistaxis, petechia, bile pigment in the urine, etc.) within one week and was examined just before death. The heart's blood showed *S. icterohemorrhagiæ* in moderate numbers. They were motile (their curves were irregular and showed lateral twitching motions or some serpentine movements). Their length varied from 9 μ to 12 μ and the width was about 0.4 μ. More organisms were seen in the emulsions of the liver and kidney. Some of the specimens were as long as 16 μ and some as short at 4 μ. The number of curves varied from 4 to 10. Inada, Ido and Hoki, and others state that the body of the organism seems to be beaded when examined under the darkfield microscope. Like other minute treponemata or spironemata, the unstained Spironema of Weil's disease is invisible under the ordinary microscope. When stained with the Giemsa, carbol fuchsin, genitan violet, or Fontana stains, the organism presents a spiral thread possessing only a few large curves with pointed extremities. There is a certain resemblance to Vincent's Spirochæta, although it is somewhat smaller and finer than the latter. A flagellum has not been demonstrated, but in a preparation stained according to the modified Fontana method,* I was able to see a delicate projection drawn out of the pointed end of the organism. Probably there is a terminal thread. It is quite astonishing, however, to find that the organisms stained by the Levaditi method appear to be very heavy, irregular forms with a few tortuous bends and blunt ends. By applying a modified technic[117] the organisms stain much more elegantly and preserve their delicate appearance.

As will be mentioned later, the *Spironema icterohemorrhagiæ* has been successfully grown on artificial media and the disease reproduced in the guinea pig by means of the pure culture.

Huebener and Reiter[80] reported early last year (1916) that they were also able to find a Spirochæta in the experimental Weil's disease in the guinea pig. The Spirochæta, designated *S. nodosa* by them, seems to be identical with the strains isolated by the Japanese investigators. As briefly referred to, Stokes has just isolated the same organisms for the Weil's disease existing in Belgium. He also succeeded in reproducing the typical disease in guinea pigs in which the organisms were demonstrated in abundance.

The report of Futaki and his associates on the finding of a spironema in the inflamed skin and lymph glands in two cases of rat-bite fever† is interesting, inasmuch as the clinical feature of this disease had already suggested to Crohn[118] its possible relation to recurrent fever. Hata and others had found

*Fix air dried film in (1) a mixture of acetic acid 8 c.c., formalin 20 c.c., and distilled water 100 c.c. for a few minutes; rinse off the fixing reagent. Cover the film with (2) a mixture of 20% tannin plus 1% phenol, and steam it over a flame for one minute; wash the film well and then immerse the slide in a 0.25% silver nitrate solution for a minute or two. After washing, cover the film once more with (2) and steam it over a flame, wash and dry.

†Symptoms: Incubation of 10 to 27 days, then chills, fever, headache and malaise. Local inflammation at the site of bite; pains in the limbs of the affected side; dark red eruptions and swollen lymph glands; 3 to 7 days fever with an afebrile interval of 2 to 3 days. Temperature curve similar to that of relapsing fever.

an effective therapeutic agent in salvarsan and mercury. These spironemata were found to be actively motile when examined by the darkfield microscope, and were successfully transmitted to the Inus monkey, guinea pig, and white rat for many generations. The organism discovered by Futaki appears to be allied to the blood spironemata of relapsing fevers. In the meanwhile this will raise an interesting question in regard to the possible existence of a spontaneous Spironema infection in rats. So far as I am aware, there is no observation on record of the finding of any pathogenic Spironema in the rat, notwithstanding the fact that this animal had been much hunted and examined by health officers for the plague bacilli, thus affording numerous opportunities to make an accidental discovery. Perhaps the finding of Futaki may open up a new field wherein to search for a hitherto undiscovered source of disease communicable to man.

VIABILITY.

There is a great deal of experimental data bearing upon the viability of various spiral organisms generally, especially upon the most widely investigated species, *Treponema pallidum*. In recording the results it is necessary to make a distinction between experiments made with uncultivated organisms and with those which have already adapted themselves to the artificial cultural conditions, in view of the fact that the latter offer a much greater resistance to certain external influences.

The free living Spirochæta lives for about a week or ten days when taken out of its natural habitat and placed in a vessel without the observance of any special precautions. On the other hand, Zuelzer[22] was able to keep various free living species of Spirochæta (plicatilis type) alive for an indefinite period of time by keeping them in a hermetically sealed vessel in which a sufficient amount of hydrogen sulphite and certain organic matters derived from stagnant water were supplied from time to time; in other words, in a culture.

The maximum time during which Cristispira can be kept alive is about two days even under favorable conditions. No culture has yet been obtained with any member of the shell fish parasites.

For Spironema it was found that the pathogenic vareties, including *S. recurrentis, S. duttoni, S. novyi, S. gallinarum*, still remain infective after a little more than 40 days when kept in a refrigerator (2°-4°C.).[18] At body temperature (37°C) complete disintegration of the organism takes place within 48 hours. No accurate data can be found regarding the saprophytic species which, it may be assumed, can remain alive much longer than their pathogenic congeners.

Of the Treponema group, *Treponema pallidum* has received most attention. Authors agree that the syphilis organism quickly becomes sluggish after being removed from the living tissues and that motility can seldom be detected in any specimen which has been maintained at 37°C. for 24 hours. On the other hand, the pallidum contained in a resected tissue (for example, a piece of chancre or rabbit's testicular syphiloma) is still found to be infective after being kept at room temperature or in a refrigerator for 48 hours or sometimes even 72 hours.* In a culture medium consisting of rabbit's plasma, a piece of rabbit's kidney and

*Isolated specimens die within 10 hours in a refrigerator (Neisser).

ascitic fluid, many pallida introduced in the form of an emulsion of rabbit's testicular syphiloma remain quite active for 3 or 4 days when kept at 37°C. under anaerobic conditions. But they do not always multiply to form a real culture. It was found that postmortem material containing *Treponema pallidum* may still be able to infect a susceptible animal when inoculated within 24 hours.[119] The organism is killed at a temperature between 50° and 55° C. maintained for twenty minutes.

The resistance and viability of cultivated strains of *T. pallidum* is much greater than that of the organisms found in the tissues. Akatsu, working in my laboratory, found that when the pallidum is isolated from a fluid culture and put in a fraction of a cubic centimeter of the same fluid, it invariably dies within 24 hours, no matter whether it be kept at 37°, 15°, or 2°C., but it survives for 5 days at 37°, 7 days at 15°, and 10 days at 2°C. when kept in 2 c.c. of the fluid. On the other hand, a small portion of a solid culture set aside in a tube remains capable of transplantation into a new medium for 48 to 72 hours at 15°C. and for 4 to 5 days at 2°C. In a quantity of about 2 c.c. of the culture, the organism remains alive as long as twenty days.

In undisturbed cultures *T. pallidum* remains alive for a considerable length of time. Thus a solid culture, set up according to the original method[120] will remain transplantable to a new medium for a period of one year uninterruptedly kept at 37°C. At 15°C. it remains alive after standing 4 or 5 months, while in a refrigerator (2°C.) it survives about 2 months. In a fluid medium consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with fluid paraffin, the organism lives about 2 to 3 months, and in a double tube method[121] about 4 months at 37°C.

T. calligyrum, T. mucosum, T. microdentium are about the same as the pallidum in regard to their resistance and viability. These organisms resist the action of the sun's rays when exposed directly for several hours (4 hours and 30 minutes) at a temperature of 30°C. (summer) and 4°C. (winter), although no growth was obtainable with material exposed for 12 hours (Akatsu).

Drying promptly kills them, that is, no growth can be obtained by transplanting the dried cultures into new media.

The thermal death points for *T. pallidum* as tested out with pure cultures are as follows:

	5 min.	10 min.	15 min.	30 min.	60 min.
45° C.	+	+	+	+	+
50° C.	+	+	+	+	+
55° C.	+	+	—	—	—
60° C.	—	—	—	—	—
65° C.	—	—	—	—	—

The above data were obtained by Akatsu and closely agree with those obtained by Bronfenbrenner,[122] who found that the several strains of *T. pallidum* were destroyed at slightly lower temperatures. It must be stated that Bronfenbrenner used isolated organisms suspended in saline or ascitic fluid, while Akatsu subjected them to the action of heat in a thin culture tube.

MICROCHEMICAL REACTION.

As mentioned elsewhere, a number of substances have been found to exert a dissolving or disintegrating action upon so-called "spirochætes" in general as well as upon certain protozoa. This phenomenon is claimed by certain authors to be decisive enough to place the spirochætes among protozoan organisms, as the majority of bacteria (pneumococcus is an exception) remain unaffected, and some can multiply freely in a saponin solution which destroys spirochætes. While a too far-reaching generalization from these observations may be avoided, these reagents nevertheless furnish us with an excellent means of studying the microchemical structure of the organisms. The following table contains a summary of all available data which, however, is very fragmentary and incomplete.

TABLE I

	TREPONEMA PALLIDUM.	SPIRONEMA RECURRENTIS.	CRISTISPIRA ANODONTÆ	SPIROCHÆTA PLICATILIS.
Saponin	10 per cent solution: 30 min., immobilized, irregular, paler. 1 hour: mostly broken up. Kills in 1:75,000 dilution.	Like pallidum when treated in 10 per cent solution.	10 per cent solution: 1-2 hours, crista fibrillar, and then indistinct.	10 per cent solution: still motile in 30 min.; longer contact makes the body shadowy, but no dissolution.
Sodium taurocholate	10 per cent solution: like the above; kills in 1:2,500 dilution.	10 per cent solution: immobile in 15 min. The outer layer shrinks into irregular masses exposing axial filament. Final disintegration.	10 per cent solution: destroyed in 15 minutes.	Same as saponin.
Sodium glycocholate	Same as sodium taurocholate.			
Sodium cholate....	Same as above, but kills in 1:5,000 dilution.			
Sodium oleate.....	10 per cent solution: Same as above, kills in 1:70,000 dilution.	Almost dissolved in 1 hour, but some may still be motile.		
Cobra lecithid.....	Kills in 1:1,000 dilution.			
Cobra venom.....	Kills in 1:1,000 dilution.			
Pepsin (0.1 in 150 c.c. of 0.3 per cent HCl)	Cells swell up in 2 hours.	.....	Slight change..	Granules appear in 2 or 3 days at 40° C., but only slight change at lower temperature.

TABLE I—(CONT'D)

	TREPONEMA PALLIDUM.	SPIRONEMA RECURRENTIS.	CRISTISPIRA ANODONTÆ.	SPIROCHÆTA PLICATILIS.
Trypsin (0.2 in 10 c.c. of 0.5 per cent Na_2CO_3)	Resist the tryptic digestion for many days.	.....	Crista, chambers and contents disappear in 24-48 hours. Membrane resistant.	Granules and axial filament made distinct in short contact. At 40° C., for 2 or 3 days, only the axial filament remains. It may break into many pieces corresponding to curves.
H_2SO_4	1 per cent solution: Immobilized immediately, shortened, granular, swollen, indistinct curves. 1 hour the same. 30 per cent solution: dissolves the organisms.	1 per cent solution: complete immobilization; many appear thinner, but forms well-preserved. 30 per cent soultion: dissolution.	30 per cent solution dissolves them.	1 per cent solution causes immediate stretching of curves, which resume their winding when adding 1 per cent KOH, or vice versa. This can be repeated many times. 30 per cent solution dissolves the spirochæta.
KOH	10 per cent solution: rendered indistinct in 30 min.; dissolution in 1 hour.	10 per cent solution: dissolves most of them in 1 hour; more resistant than the pallidum.	1 per cent solution destroys Crista, membrane resists 10 per cent, but dissolved in 30 per cent with heat.	1 per cent solution dissolves granules, 2-30 per cent destroy spirochæta, axial filament most resistant. Treatment with absolute alcohol accelerates the dissolving power of KOH.
Na_2CO_3	1 per cent solution: immobilized, but no morphological changes.	1 per cent solution; immobilized, slightly granular, but well preserved.	.....	1 per cent solution: no effect on plasma, dissolves granules.

As will be noticed in Table I, certain reagents demonstrate the existence of a resistant membrane in Cristispira, a trypsin resistant axial filament in Spirochæta, and a shadowy sheath (?) as well as an axial spiral filament in Spironema and Treponema. As in the case of Spirochæta, no true dissolution of Spironema (both gallinarum and recurrentis) or Treponema was effected by the saponin, but after several hours' contact they were shrivelled and broken up into irregular pieces.

Resistance to Disinfectant and Chemotherapeutic Agents.—Attempts to determine the resistance of various "spirochætes" are not lacking, but no satistactory and accurate results were to be expected from the experiments in which their death point had to be determined through the intermediary of susceptible animals. Since the successful cultivation of different "spirochætes" has been effected, it has become possible to determine the effect of different chemicals. The following chart shows a summary of the results obtained in two independent series of experiments by the use of common disinfectants.

RESISTANCE TO CHEMICALS.

At 37° C.	
Lugol kills in	1:3 dil.; 1:5-1:10 in 15 min.; 1:50 not in 1 hour.
Bichloride of mercury kills in	1:5,000 dil.; 1:10,000 in 15 min.; 1:50,000 in 30 min.; 1:100,000 not in 1 hour.
At room temperature	
Phenol kills in	1:200; 1:1,000 in 30 min.; 1:5,000 not in 1 hour.
Lysol kills in	1:1,000; 1:5,000 not in 1 hour.
Formalin kills in	1:200; 1:500 in 15 min.; 1:1,000 not in 1 hour.
Potassium permanganese kills in	1:1,000; 1:5,000 in 15 min.; 1:10,000 not in 1 hour.

Turning our attention to the chemotherapeutic agents it is scarcely necessary to remark that, thanks to the pioneer work of Ehrlich and his collaborators, especially to his contribution to our chemical treatment of spironematoses and trypanosomiases, a new field of scientific research has been inaugurated. Thus Morgenroth initiated a chemotherapy for bacterial diseases by discovering various quinin derivatives as a specific for pneumococcus. Flexner and Clark, with the collaboration of Jacobs and Heidelberger,[123] made an extensive series of experiments in order to discover an effective chemical compound to combat poliomyelitis, wherein they obtained some encouraging results. In their early work they had employed numerous new derivatives of urotropin (hexamethylenetetramine) as this substance was known to penetrate into the intrathecal space. The work has since been extended to various bacterial infections[124, 125, 126, 127] as well as trypanosomiasis and spironematosis (Brown and Pearce) with the use of additional new arsenic and mercurial compounds. While I do not wish to assert that the therapeutic effect of a chemical compound has any direct relation to the latter's disinfecting or sterilizing power against the causative agent *in vitro*, it was nevertheless thought of interest to find out how these new compounds, including various derivatives of urotropin, arsenic, and mercury, would behave in relation to the various species of Spironema and Treponema in cultures.

It is a well known fact that atoxyl, arsacetin or arsenophenolglycin, and even salvarsan, attack the trypanosomes and spironemata only after being introduced into the body, where they undergo reduction and produce a highly parasitotropic component. Yet, as will be shown in the following table, salvarsan is by no means inactive *in vitro* against *T. pallidum*. It is a fairly powerful treponemicide. Hence it is not without interest to study these compounds *in vitro* and then, when completed, compare the results with their therapeutic effects *in vivo*. The test tube determination of the germicidal property of these substances should form a part of our knowledge in perfecting chemotherapy. With the cooperation of Dr. Jacobs, who is in charge of the preparation of chemotherapeutic agents at the Rockefeller Institute, the following compounds were tested on cultivated strains of *T. pallidum in vitro* with the results indicated in the tables. A fuller report will be made later by Dr. Akatsu.

TABLE II.

No.	Preparation.	Concentration sufficient to kill T. pallidum.	Concentration which no longer kills T. pallidum.
9.	*p*-Bromobenzylhex. chloride	1 : 1,000	1 : 2,500
16.	*o*-Xylylenedi-hex. chloride	1 : 2,500	1 : 5,000
19.	2-Nitro-3,4-Dimethoxybenzylhex. chloride	1 : 2,500	1 : 5,000
21.	1-(ω-chlorobenzyl)-2-oxy-3-naphthoic methyl ester)+hex.	1 : 2,500	1 : 5,000
28.	5-Chloromethylvanillin+hex.	1 : 750	1 : 1,000
29.	5-Chloromethylsalicylic acid+hex	1 : 2,500	1 : 1,500
40.	*p*-iodobenzylbromide+hex.	1 : 750	1 : 1,000
46.	*o*-nitrobenzylchloride+hex.	1 : 250	1 : 500
47.	*p*-nitrobenzylhex. chloride	1 : 750	1 : 1,000
50.	Methylhex. iodide	1 : 100	1 : 250
84.	Chloroacetamide+hex.	1 : 1,000	1 : 2,500
86.	Oxymethylchloroacetamide+hex.	1 : 250	1 : 500
90a.	Ethyl bromoacetate+hex	1 : 1,000	1 : 2,500
96.	Chloroacetylaniline+hex.	1 : 1,000	1 : 2,500
97.	β-acetoxy-α-chloroacetylnaphthobenzylamine+hex.	1 : 1,000	1 : 2,500
102.	Chloroacetyl-α-naphthylamine+hex.	1 : 500	1 : 750
107.	Chloroacetylbenzylamine+hex.	1 : 500	1 : 750
109.	Chloroacetyl-β-naphthylamine+hex.	1 : 1,000	1 : 2,500
111.	*o*-Methylchloroacetylbenzylamine+hex.	1 : 2,500	1 : 5,000
112.	Chloroacetyl-*p*-aminobenzoic ethyl ester+hex	1 : 1,000	1 : 2,500
114.	Chloroacetylurea+hex.	1 : 1,000	1 : 2,500
121.	Phenoxyethylhex. bromide	1 : 250	1 : 500
122.	*p*-Bromochloroacetylaniline+hex.	1 : 2,500	1 : 5,000
126.	Chloroacetylaminoazotoluene+hex.	1 : 250	1 : 500
134.	Chloroacetyl-*p*-anisidine+hex.	1 : 2,500	1 : 5,000
138.	Chloroacetylphenylhydrazine+hex.	1 : 750	1 : 1,000
142.	Chloroacetothylamide+hex.	1 : 1,000	1 : 2,500
146.	Menthyl bromoacetate+hex	1 : 750	1 : 1,000
147.	Bromoethylphthalimide+hex.	1 : 1,000	1 : 2,500
148.	*p*-nitrobenzoic bromoethyl ester+hex	1 : 250	1 : 500
150.	Bromoethyl benzoate+hex	1 : 500	1 : 750
158.	β-Iodopropionyl-*o*-anisidine+hex.	1 : 1,000	1 : 5,000
163.	*p*-ethoxyphenyl bromomethyl ketone+hex	1 : 500	1 : 750
164.	Chloroacetyl-ψ-cumidine+hex.	1 : 2,500	1 : 5,000
168.	*p*-Acetamino-ω-bromoacetophenone+hex.	1 : 750	1 : 1,000
171.	m-Chloroacetylaminomethylbenzamide+hex.	1 : 1,000	1 : 2,500
172.	m-Chloroacetyl-α, α,-phenylbenzylhydrazine+hex	1 : 2,500	1 : 5,000
174.	Chloroacetyl-aminoethyl anisate+hex	1 : 500	1 : 750
204.	3-(ω Bromoacetyl) quinaldine+hex	1 : 2,500	1 : 5,000
218.	Tribromo-*p*-cresyl bromoethyl ether+hex	1 : 2,500	1 : 5,000
219.	Chloroacetyl-p-aminoleucomolachite green+hex.*	1 : 5,000	1 : 7,500
229.	Chloroacetyl-*p*-aminobenzeneazo-p′-dimethylaniline + hex.*	1 : 500	1 : 750
232.	*p*-Chloroacetylaminobenzeneazo-p′-diethylaniline+hex.	1 : 1,000	1 : 2,500
234.	α-naphthyl bromoethyl ether+hex	1 : 500	1 : 750
239.	*o*-Acetaminophenyl bromoethyl ether+hex	1 : 1,000	1 : 2,500
242.	*p*-chloroacetylaminodiethylaniline+hex.	1 : 1,000	1 : 2,500
244.	Hex.+chloroacetylaminoethyl *p*-nitrobenzoate	1 : 2,500	1 : 5,000
249.	Chloroacetyl-*p*-aminodipropylaniline+hex.*	1 : 500	1 : 750
252.	Chloroacetyl-*p*-diaminotetraethyl-p′, p″, -diaminotriphenyl-methane+hex.	1 : 1,000	1 : 2,500
253.	Chloroacethyldiethylamine+hex.	1 : 1,000	1 : 2,500
255.	*p*-Cyanobenzylhex. chloride	1 : 1,000	1 : 2,500
257.	Chloroacetyl-*o*-aminophenyl benzoate+hex	1 : 1,000	1 : 2,500
261.	Chloroacetyltriphenylmethylamine+hex.	1 : 1,000	1 : 2,500
262.	Chloroacetylleucoauramine+hex. (*?)	1 : 1,000	1 : 2,500
263.	Chloroacetylaminoethyl *o*-nitrobenzoate+hex	1 : 1,000	1 : 2,500
267.	Chloroacetylaminoethyl β-naphthoate+hex	1 : 2,500	1 : 5,000
271.	Chloroacetyl-N-phenylaminoethyl-*p*-nitrobenzoate+hex.	1 : 1,000	1 : 2,500
272.	*m*-Acetamino-*p*-tolyl ω-iodoethyl ketone+hex	1 : 5,000	1 : 7,500
273.	Chloroacetylethylaminoethyl *p*-nitrobenzoate+hex	1 : 1,000	1 : 2,500
278.	α, β-Diphenylchloroacetylamino-ethanol+hex	1 : 250	1 : 500

TABLE II.—(CONT'D)

No.	Preparation.	Concentration sufficient to kill T. pallidum.	Concentration which no longer kills T. pallidum.
280.	Chloroacetyl-*m*-aminoacetophenone+hex.	1 : 1,000	1 : 2,500
282.	α-Phenyl-α-oxy-β-chloroacetylaminoethane+hex.	1 : 1,000	1 : 2,500
283.	*p*-nitrobenzoylaminoisopropyl chloroacetate+hex.	1 : 500	1 : 750
288.	Iodopropanol+hex.	1 : 500	1 : 750
289.	2-Chloroacetylamino-3-oxy-3-methylbutane+hex.	1 : 2,500	1 : 5,000
291.	Chloroacetyl-*o*-methylphenoxyethylamine+hex.	1 : 1,000	1 : 2,500
293.	Chloroacetyl-β-amino-δ-butanol+hex.	1 : 250	1 : 500
298.	β-Phenyl-β-oxy-δ-chloroacetylaminopropane+hex.	1 : 1,000	1 : 2,500
301.	β-Naphthyl bromethyl ether+hex.	1 : 1,000	1 : 2,500
303.	2-oxy-3, 5-dibromobenzyl bromide (+?)+hex.	1 : 1,000	1 : 2,500†
308.	Chloroacetyl-*m*-iodoaniline+hex.	1 : 750	1 : 1,000
309.	Chloroacetyl-5-iodo-*o*-toluidine+hex.	1 : 750	1 : 1,000
M1.	(4-[p-oxybenzeneazo]-phenylmercuric acetate)	1 : 50,000	1 : 75,000
M4.	[o]oxybenzylideneamino] phenylmercuric acetate+	1 : 50,000	1 : 75,000
M7.	1-Amino-2-[p-naphthaleneazophenylmercuric acetate]-5-sulfonic acid	1 : 25,000	1 : 50,000

Hex.=Hexamethylenetetramine.
*=Grind up in a mortar with a little water and add N/10 HCl carefully until dissolved.
†=Treat as above, using N/10 NaOH instead of HCl.

TABLE III

Names of substances	Concentration sufficient to kill T. pallidum.	Concentration in which T. pallidum survived.
Phenol	1 : 2,500	1 : 5,000
Formalin	1 : 750	1 : 1,000
Lysol	1 : 5,000	1 : 7,500
Sublimate	1 : 100,000	1 : 500,000
Salvarsan	1 : 7,500	1 : 10,000
Neosalvarsan	1 : 2,500	1 : 5,000
Atoxyl		
Sodium iodide	1 : 10	1 : 25
Potassium iodide	1 : 10	1 : 25
Lugol's solution	1 : 75	1 : 100
Iodox. benz. acid	1 : 500	1 : 1,000
Trypozofrol	1 : 25,000	1 : 50,000
Nectrypozofrol	1 : 250	1 : 1,000
Sodium cholate	1 : 5,000	1 : 7,500
Sodium glycocholate	1 : 2,500	1 : 5,000
Sodium taurocholate	1 : 2,500	1 : 5,000
Sodium oleinicum	1 : 70,000	1 : 50,000
Saponin	1 : 75,000	1 : 100,000
Cholesterin	No action	No action
Cobra lecithid	1 : 1,000	1 : 5,000
Cobra venom	1 : 1,000	1 : 5,000

TABLE IV

Names of organisms.	Preparation M1.			Preparation No. 253.	
	1/10,000	1/25,000	1/50,000	1/1,000	1/2,500
T. pallidum, heavy type	..	—	+	—	+
T. pallidum, thin type	—	+	..	—	+
T. calligyrum	..	—	+	—	+
T. mucosum	..	—	+	—	+
T. microdentium	..	—	+	—	+
S. refringens	..	—	+	—	+

Table II gives a general survey of these compounds, while Table III puts down the strengths of various well known disinfectants and chemicals for the sake of comparison. Table IV gives the resistance of several culture strains of pallidum and other allied species to the action of two different new compounds.

As briefly mentioned the spironemicidal (or treponemicidal) power of salvarsan and neosalvarsan is alleged to increase considerably when introduced into the living body. In a series of experiments,[122] it was found that by allowing a sterile extract of freshly removed rabbit's liver or defibrinated blood of the same animal to act upon neosalvarsan for three hours at 37°C. the germicidal power of this drug increased from 1:1,000 to 1:2,000 in the case of the liver extract, and from 1:1,000 to 1:5,000 in the case of the blood. The addition of boiled extract had no such activating effect.

Acquisition of Increased Resistance to Drugs.—It will be recalled here that the failure of chemotherapy in trypanosomiasis in man and animal is partly due to the production of so-called drug-fast strains of various trypanosomes after the latter have on several occasions been subjected to the action of certain arsenic compounds. These organisms will be destroyed to a great extent by the first injection of the drugs, but if there remain a few which have resisted the first medication, they will multiply and the animal will once more be infested with the organisms. The offspring is more resistant to the action of the same drug than the preceding generation. A large dose of the medicament is necessary to destroy the organisms and to overcome this increased resistance. But as a matter of fact, the increased resistance of the organism to the drug is relatively much greater than that of the infected hosts and the limit will soon be reached beyond which the quantity of the drug cannot be further increased without seriously affecting the infected man or animal.

Experiments of this nature have been made with atoxyl, arsacetin, arsenophenylglycin, etc. To employ Ehrlich's terms, the organotropic affinities of these drugs were so close to the parasitotropic, that it was impossible to employ a sufficient quantity to completely sterilize the infected body, since the administration of such a quantity would mean death or the serious impairment of some of the functions. Ehrlich's conception of a specific chemotherapy was based upon the fact that different cell groups are provided with their characteristic receptor apparatus (chemoceptor), to which a given chemical molecule attaches by means of its side chains. Thus, for trypanosomes there are certain receptors which will fit in with a certain atom complex of atoxyl, arsacetin, etc., while of the infected hosts the organs show much less affinity for them. In developing chemotherapy for syphilis, Ehrlich finally evolved a compound in which the spironematropic atom complexes were far more in excess than the organotropic groups. This compound, as is universally known, is dioxydiamidoarsenobenzol, better known as salvarsan. According to Hata[128] the ratio of the dosis curativa and dosis tolerata of this compound is 1:3 for mice and rats infected with Spironema recurrentis, and 1:58 for chickens with *S. gallinarum*, while in the case of experimental chancre in rabbits it is between 1:7-1:10. In these animals Ehrlich's *Therapia sterilisans magna* was achieved as also in cases of relapsing fevers in man. In human syphilis, however, in spite of the most powerful spironemicidal

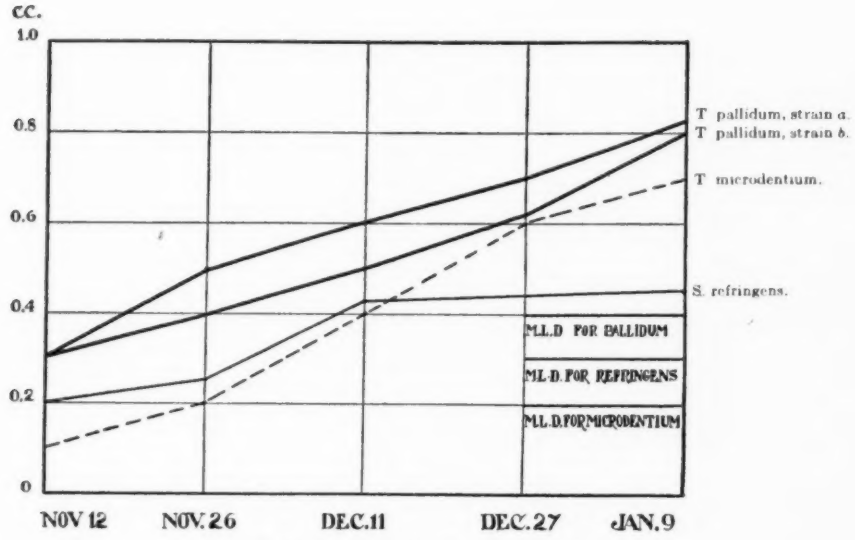
action, his original aim to sterilize the syphilitic body with a single injection of a large dose was not uniformly attained.

Yet there is no doubt that a prompt administration of salvarsan in a sufficient dose during the early stage of infection sterilized the patients, as was evidenced by the increased instances of permanent abortion of the infection and of reinfection after the salvarsan treatment. On the other hand, we are also confronted with repeated recidives in certain patients. We often hear of mercury-resistant as well as salvarsan-refractory cases. It has been known for some time that *Spironema recurrentis* as well as *Spironema duttoni* produces an arsenic-fast strain in mice or rats when the latter are treated with atoxyl, arsacetin, etc. In this respect these spironemata resemble trypanosomes. Marks[129] once considerably raised the resistance of a bacteria to arsenious acid by allowing it to gradually accustom itself to the action of this chemical in test tube cultures. It, therefore, seems not at all improbable that Spironema as well as Treponema become more resistant to the parasitotropic effect of arsenic compounds and possibly of mercurial salts, not only *in vivo*, but *in vitro*. Akatsu carried out a number of experiments in my laboratory in which he has apparently succeeded in raising to many times their original degree the resistance of the Treponema group to salvarsan, neosalvarsan, and bichloride of mercury. The experiments were carried out with cultures of these organisms, the general plan being to cultivate the organisms in media containing these substances in a concentration just short of that required to suppress the growth completely, and to make subcultures from it into new media containing somewhat greater quantities of the chemicals than the preceding series. In the present experiments fluid cultures consisting of ascitic fluid and a piece of fresh rabbit's kidney covered with a layer of liquid paraffin were employed. Subcultures from one medicated culture to another were made at two weeks' intervals, during which time the general condition of the cultures could be estimated. As mentioned above, subcultures are made from tubes still showing numerous actively motile organisms. It is difficult to carry on the culture if one attempts to make a subculture in which too much medicament is present to give a fairly good growth, since no growth will be obtained in a subculture which has been inoculated with a poor culture arrested in its development by an excess of the drugs.

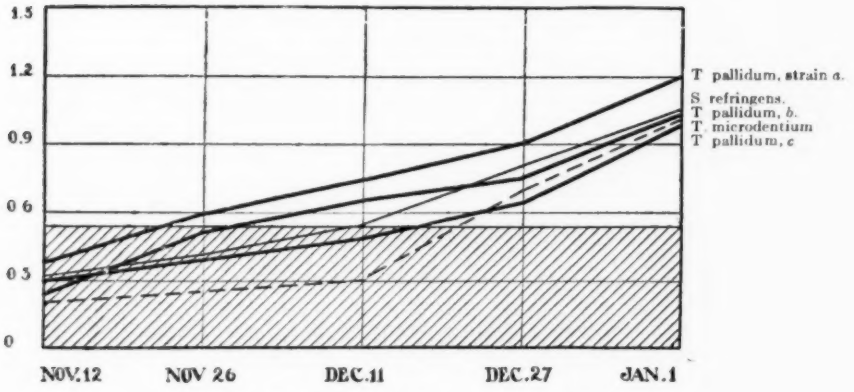
In order to suppress the growth of various treponemata which had not previously been in contact with these compounds, the following doses were found necessary in a total volume of 5 c.c. of the culture medium. The solutions of salvarsan and neosalvarsan were 1:10,000 dilution in water, and bichloride of mercury 1:1,000 dilution. Salvarsan was neutralized with NaOH, as usual.

	SALVARSAN.	NEOSALVARSAN.	$HgCl_2$.
Refringens	0.4 c.c.	0.6 c.c.	0.02 c.c.
Pallidum (two strains)	0.375 c.c.	0.5 c.c.	0.02 c.c.
Microdentium	0.2 c.c.	0.3 c.c.	0.02 c.c.

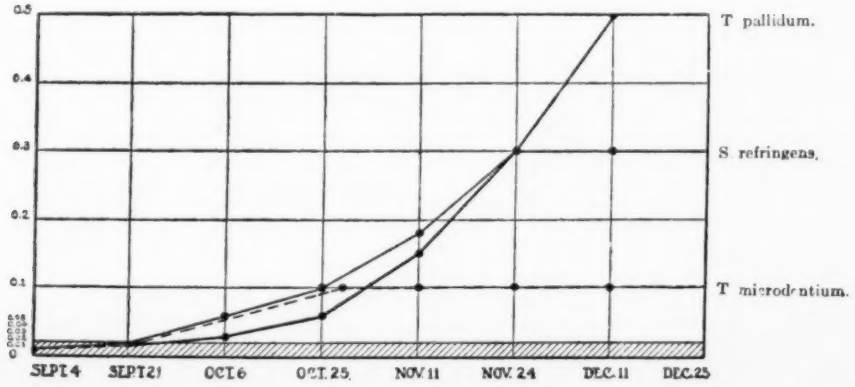
It will be seen from the charts that the resistance of different species of Treponema and also of different strains of the same species (*T. pallidum*) seemed to



1:10,000 dilution of salvarsan in 5 c.c. of medium.



1:10,000 dilution of neosalvarsan in 5 c.c. of medium.



1:1000 dilution of bichloride of mercury in 5 c.c. of medium.

increase gradually until at the end of ten weeks (five transfers), they were still able to grow very well in a medium which contained several (2 to 3.5) times the quantity of the arsenic compounds originally sufficient to restrain their growth completely. In case of bichloride of mercury the increased rate of tolerance was still more striking within a certain limit of concentration, but there was no further increase in resistance when the medium contained more than 0.5 c.c. of the 1:1000 dilution of this salt. The tissues which usually remain fleshy pink in color for several days became quickly discolored and a dirty grayish black when mixed with the above concentration of $HgCl_2$.

The question of the duration of the acquired resistance to the drugs has not yet been studied a sufficiently long time to draw any conclusions, but the resistance has remained unmodified for at least three generations. It may be mentioned that *Spironema recurrentis* was carried through two generations in mice without undergoing any change in its acquired drug fastness.

BIBLIOGRAPHY.

[1]Ehrenberg, Ch.: Die Infusionstierchen als vollkommene Organismen, Leipzig, 1838.
[2]Schaudinn, F.: Generations— und Wirtswechsel bei Trypanosomen und Spirochæten, Arb. a. d. k. Gsndhtsamte, 1904, xx, 387.
[3]Sergent, Ed., and Sergent, Ét.: Les hématozoaires d'oiseaux, Ann. de l'Inst., Pasteur, 1907, xxi, 251.
[4]Mayer, M.: Ein Halteridium und Leukocytozoon des Wildkauzes, Arch. f. Protistenk., 1911, xxi, 232.
[5]Mathis, C., and Léger, M.: Parasitologie et Pathologie humaines et animales au Tonkin, Paris, 1911.
[6]Schaudinn, F., and Hoffmann, E.: Vorläufiger Bericht über das Vorkommen von Spirochæten in syphilitischen Krankheitsprodukten und bei Papillomen, Arb. a. d. k. Gsndhtsamte, 1905, xxii, 527.
[7]Schaudinn, F.: Zur Kenntnis der Spirochæte pallida, Deutsch. med. Wchnschr., 1905, xxxi, 1665.
[8]Schaudinn, F., and Hoffmann, E.: Über Spirochæta pallida bei Syphilis und die Unterschiede dieser Form gegenüber anderen Arten dieser Gattung. Berl. klin. Wchnschr., 1905, xiii, 673.
[9]Schaudinn, F.: Korrespondenz, Deutsch. med. Wchnschr., 1905, xliii, 1728.
[10]Vuillemin, P.: Sur la dénomination de l'agent de la syphilis, Compt. rend. Acad. d. sc., 1905, cxl, 1567.
[11]Stiles, C. W., and Pfender, C. A.: The Generic Name (Microspironema) of the Parasite of Syphilis, Am. Med., 1905, x, 936.
[12]Swellengrebel, N. H.: Neuere Untersuchungen über die vergleichende Cytologie der Spirillen und Spirochæten, Centralbl. f. Bakteriol., Abt. 1, 1909, xlix, 529.
[13]Sambon: Manson's Tropical Diseases, Wm. Wood & Co., N. Y., Ed. 4, 1907, 833.
[14]Schilling: Die neueren Fortschritte auf dem Gebiete der pathogenen Protozoen, Centralbl. f. Bakteriol., Abt. 1, Ref. Beil., 1908, xlii, 80.
[15]Klebs, G.: Flagellatenstudien, Ztschr. wissensch. Zool., 1892, lv, 265.
[16]Gross, J.: Cristispira nov. gen., ein Beitrag zur Spirochætenfrage, Mitt. a. d. zool., Station zu Neapel, 1910, xx, 1.
[17]Perrin, A.: Researches on the Life History of Trypanosoma Balbianii (Certes), Arch. f. Protistenk., 1906, vii, 131.
[18]Novy, G., and Knapp, R. E.: Studies in Spirillum Obermeieri and Related Organisms, Jour. Infect. Dis., 1906, iii, 291.
[19]Schellack, C.: Studien zur Morphologie und Systematik der Spirochæten aus Muscheln, Arb. a. d. k. Gsndhtsamte., 1909, xxx, 379.
[20]Gross, J.: Über Systematik, Struktur und Fortpflanzung der Spironemacea, Centralbl. f. Bakteriol., Abt. 1, 1912, lxv, 83.
[21]Gross, J. Zur Nomenklatur der Spirochæta pallida Schaudinn und Hoffmann, Arch. f. Protistenk., 1911, xxiv, 109.
[22]Zuelzer, M.: Über Spirochæta plicatilis Ehrenberg und deren Verwandtschaftsbeziehungen, Arch. f. Protistenk., 1911, xxiv, 1.

[23]Gonder, R.: Die Stellung der Spirochæten unter den Protisten, Centralbl. f. Bakteriol., 1909, xlix, 190.
[24]Dobell, C. C.: On Cristispira veneris Nov. Spec. and the Affinities and Classification of Spirochætes, Quart. Jour. Micr. Sc., 1910-1911, lvi, 507.
[25]Dobell, C. C.: On the Systematic Position of the Spirochætes, Proc. Roy. Med. Soc., 1912, lxxxv, 186
[26]Dobell, C. C.: Researches on the Spirochætes and Related Organisms, Arch. f. Protistenk., 1912, xxvi, 117.
[27]Hoelling, A.: Spirillum Giganteum und Spirochæta Balbiani, Centralbl. f. Bakteriol., Abt. 1, 1907, xliv, 665.
[28]Hoelling, A.: Vergleichende Untersuchungen über Spirochæten und Spirillen, Arch. f. Protistenk., 1911, xxiii, 101.
[29]Fantham, H. B.: Spirochæta Balbianii (Certes) and Spirochæta anodonta (Keysselitz), Their Structure, Movements, and Affinities, Quart. Jour. Micr. Sc., 1908, lii, 1.
[30]Fantham, H. B.: Some Researches on the Life Cycle of Spirochaetes, Ann. Trop. Med. and Parasit., 1911, v, 479.
[31]Swellangrebel, N. H.: Sur la cytologie comparée des spirochètes et des spirilles, Ann. de l'Inst. Pasteur, 1907, xxi, 448; and Compt. rend. Soc. de biol., 1907, lxii, 213.
[32]Bosanquet, W. C.: Note on the Structure and Development of Spirochæta Anodontæ Keysselitz, Quart. Jour. Micr. Sc., 1911, lvi, 387.
[33]Bosanquet, W. C.: Spirochætes, Philadelphia, 1911.
[34]Doflein, F.: Lehrbuch der Protozoenkunde, Jena, 1912.
[35]Fantham, H. B., and Porter, A.: The Modes of Division of Spirochaeta recurrentis and Spirochæta duttoni as Observed in the Living Organisms, Proc. Roy. Soc. Biol., 1909, lxxxi, 500.
[36]Schaudinn, F.: Zur Kenntnis der Spirochæta pallida und andere Spirochæten. Nachlass., Arb. a. d. k. Gsndhtsamte., 1907, xxvi, 11.
[37]Borrel, A.: Cils et division transversale chez le spirille de la poule, Compt. rend. Soc. de biol., 1906, lx. 138.
[38]Zettnow, E.: Geisseln bei Hühner- und Recurrensspirochäten, Deutsch. med. Wchnschr., xxxii, 376.
[39]Hoffmann, E., and Prowazek, S. V.: Balanitis und Mundspirochäten, Centralbl. f. Bakteriol, Abt. 1, 1906, xli, 741.
[40]Prowazek, S. v.: Vergleichende Spirochätenuntersuchungen, Arb. a. d. k. Gsndhtsamte, 1907, xxvi, 22.
[41]Landsteiner, K., and Mucha, V.: Zur Technik der Spirochätenuntersuchung, Wien. klin. Wchnschr., 1906, xlv, 1349.
[42]Bütschli, O.: Über den Bau der Bakterien und verwandte Organismen, Leipzig, 1890.
[43]Bütschli, O.: Bemerkungen über Cyanophyceen und Bacteriaceen, Arch. f. Protistenk., 1912, i, 41.
[44]Gross, J.: Sporenbildung bei Cristispira, Arch. f. Protistenk., 1913, xxix, 279.
[45]Gonder, R.: Spironemacea (Spirochäten), Prowazek's Handbuch der pathogenen Protozoen, 1914, 671.
[46]Cantacuzène: Sur un spirochète thermophile des eaux de Dax, Compt. rend. Soc. de biol., 1910, lxviii, 75.
[47]Certes: Trypanosoma balbianii, Bull. Soc. zool. franc., 1882, vii, 347; Ibid., 1891, xvi, 95.
[48]Laveran, A., and Mesnil, V.: Sur la nature bactérienne du prètendu Trypanosoma des huitres, Compt. rend. Soc. de biol., 1901, liii, 881.
[49]Keysselitz, G.: Beschreibung von Spirochæta anodontæ nov. sp., Arb. a. d. k. Gsndhtsamte., 1906, xxiii. 566.
[50]Keysselitz, G.: Studien ueber Protozoen, Arch. f. Protistenk., 1907, xi, 334.
[51]Mackinnon, D. L.: Observations on the Division of Spirochætes, Parasitology, 1909, ii, 267, 281.
[52]Vlès, F.: Sur la structure et les affinités de Trypanosoma balbianii, Compt. rend. Soc. de biol., 1906, lxi, 408.
[53]Borrel, A., and Cernovodeanu: Membrane ondulante du Spirochæte balbianii. Compt. rend. Soc. de biol., 1907, lxii, 1102.
[54]Gross, J.: Über freilebende Spironemaceen, Mitt. a. d. zool., Station zu Neapel, 1910-13, xx. 188.
[55]Flexner, S.: Spirochæta (Treponema) pallida and Syphilis, Jour. Exper. Med., 1907, ix, 464.
[56]Gonder, R.: Studien über die Spirochäte aus dem Blute von Vesperugo Kuhlii, Arb. a. d. k. Gsndhtsamte., 1908, xxvii, 406.
[57]Noguchi, H.: A Method for Pure Cultivation of Pathogenic Treponema pallidum, Jour. Exper. Med., 1911, xiv, 99.
[58]Prowazek, S. v.: Morphologische und entwicklungsgeschichtliche Studien über Hühnerspirochäten, Arb. a. d. k. Gsndhtsamte., 1906, xxiii, 554.

[59]Fraenkel, C.: Geisselfäden an den Spirillen des Rekurrens- und Zeckenfiebers, Centralbl. f. Bakteriol., Abt. 1, 1908, xlvii, 349, 471.
[60]Szécsi, St.: Lucidol, ein neues Fixiermittel, Deutsch. med. Wchnschr., 1913, xxxiii, 1584.
[61]Galli-Valerio, B.: Note de La Methode de Casares-Gil pour la coloration des cils des bacteries, Centralbl. f. Bakteriol., Abt. 1, 1915, lxxvi, 233.
[62]Nakano, H.: Über die Reinzüchtung der Spirochæta pallida, Deutsch. med. Wchnschr., 1912, xxxix, 1332.
[63]Krysztalowicz, F., and Siedlecki, M.: Contribution à l'étude de la structure et du cycle évolutif de Spirochæte pallida Schaudinn, Bull. Acad. Sc. Krakau, 1905, No. 9, 713.
[64]Meirowsky, E.: Studien über die Fortpflanzung von Bakterien, Spirillen und Spirochæten, Berlin, 1914.
[65]Balfour, A.: Spirochætosis of Sudanese fowls, 3. Rep. of the Wellcome Res. Lab., 1908, 35-58, and 4. Rep. of the Wellcome Labs., Khartoum, 1911, 76-107.
[66]Balfour, A.: Notes on the Life Cycle of the Sudan Fowl Spirochæte, Internat. Congr. of Med., London, 1913, xxi, 275.
[67]Leishman, W. B.: Observations on the Mechanism of Infection in Tick Fever and on the Hereditary Transmission of Spirochaeta Duttoni in the Tick, Lancet, 1910, cxxviii, 11-14; and Tr. of Soc. Trop. Med. and Hyg., 1910, iii, 77-95.
[68]Blanc, G. R.: Les spirochètes contribution à l'étude de leur évolution chez les ixodidae, Thèses de Paris, 1910-11, No. 432.
[69]Fantham, H. B.: The Granule Phase of Spirochætes, Ann. Trop. Med. and Parasit., 1914, viii, 471.
[69a]Nuttall, G. H. F.: Spirochætosis, Harvey Lect., Phila. and London, 1912-13, 28-43.
[70]Hindle, E.: On the Life Cycle of Spirochæta Gallinarum, Ann. Trop. Med. and Parasit., 1911, iv, 463.
[71]Marchoux, E., and Couvy, E.: Argas et Spirochètes, Ann. de l'Inst. Pasteur, 1913, xxvii, 450, 620; Bull. Soc. Path. Exot., 1912, v, 63, 796.
[72]Gleitsmann: Beitrag zur Entwicklungsgeschichte der Spirochæten (Borrelien), Centralbl. f. Bakteriol., Abt. 1, 1913, lxviii, 31.
[73]Todd, J. L., and Wolbach, S. B.: The Filterability of Spirochæta duttoni, Jour. Med. Research, 1914, xxx, 27.
[74]Obermeier, O.: Vorkommen feinster, eine Eigenbewegung zeigender Fäden im Blute von Recurrenskranken, Centralbl. f. d. med. Wissensch., 1873, 145.
[75]Carter, V.: Spirillum Fever, London, 1882.
[76]Dutton, J. E., and Todd, J. L.: Note on Morphology of Spirochæta duttoni, Lancet, 1907, ii, 1523.
[77]Schellack, C.: Morphologische Beiträge zur Kenntnis der europäischen, amerikanischen und afrikanischen Rekurrensspirochæta, Arb. a. d. k. Gsndhtsamte, 1907, xxvii, 364.
[78]Sergent, E., and Foley, H.: Récherches sur la fièvre récurrente et son mode de transmission, dans une épidémie algérienne, Ann. de l'Inst. Pasteur, 1910, xxiv, 337.
[79]Inada, Ido, Hoki, Kaneko and Ito: Weil's Disease (Spirochæta icterohemorrhagica), Jour. Exper. Med., 1916, xxiii, 377.
[80]Huebener and Reiter: Beiträge zur Aetologie der Weil'schen Krankheit., Deutsch. med. Wchnschr., 1915, xli, 1275.
[81]Marchoux, E., and Salimbeni, A.: La spirillose des poules, Ann. de l'Inst. Pasteur, 1903, xvii, 569.
[82]Sacharoff: Spirochæta anseruna et la septicémie des oies, Ann. de l'Inst. Pasteur, 1891, v, 564.
[83]Theiler, A.: Spirillosis of Cattle, Jour. Comp. Path. and Therapy, 1903, xlvii, 55.
[84]Breinl, A., and Kinghorn, A.: Preliminary Note on a New Spirochaeta Found in a Mouse, Lancet, London, 1906, ii, 651.
[85]Wenyon, C. M.: Spirochætosis of Mice Due to Spirochæta Muris, n. sp. in the Blood, Jour. Hyg., Cambridge, 1906, vi, 580.
[86]Carter, V.: Note on the Occurrence of a Minute Blood Spirillum in an Indian Rat, Sc. Mem. by Med. Officers of the Army of India, 1887, iii, 45.
[87]Löwenthal, W.: Beitrag zur Kenntnis der Spirochaeten, Berl. klin. Wchnschr., 1906, xliii, 283.
[88]Gonder, R.: Spirochäten aus dem Darmtraktus von Pinna: Spirochæta pinnæ nov. spec. and Spirochæta hartmanni nov. spec., Centralbl. f. Bakteriol., Abt. 1, 1908, xlvii, 491.
[89]Vincent, H.: Note zur le microbe de la pourriture d'Hôpital, Bull. de l'acad. de méd., 1896, xxxv, 87; and Sur. L'Étiologie et sur les Lésions anatomo-Pathologique, etc., Ann. de l'Inst. Pasteur, 1896, x, 488.
[90]Vincent, H.: Symptomatologie et diagnostic de l'angine à spirilles et bacilles fusiformes (Angine de Vincent), Lancet, London, 1905, 1260.

[91]Veszprémi, D.: Züchtungs- und Tierversuche mit Bacillus fusiformis, Spir. gracilis und Cladothrix putridogenes, Centralbl. f. Bakteriol., Abt. 1, 1907, xliv, 332, 408, 515, 648, and xlv, 15.
[92]Prowazek, S. v.: Vergleichende Spirochätesuntersuchungen, Arb. a. d. k. Gsndhtsamte., 1907, xxvi, 23.
[93]Mulzer, P.: Über das Vorkommen von Spirochæten bei Syphilis und anderen Krankheitsprodukten, Berl. klin. Wchnschr., 1905, xlii, 1144.
[94]Fantham, H. B.: Spirochæta bronchialis, Castellani, 1907, together with Remarks on the Spirochætes of the Human Mouth, Ann. Trop. Med. and Parasit., 1915, ix, 391.
[95]Noguchi, H.: Pure Cultivation of Spirochæta phagedenis (n. s.), Jour. Exper. Med., 1912, xvi, 261.
[96]Castellani, A.: Further Observations on Parangi (yaws), Brit. Med. Jour., 1905, ii, 1330.
[97]Cleland, J. B.: Note on Spirochætes in Castration Tumors of Pigs, Parasitology, 1908, i, 218.
[98]King, W. E., and Bæslack, F. W.: Studies on the Virus of Hog Cholera, Jour. Infect. Dis., 1913, xii, 39.
[99]King, W. E., Drake, R. H., and Hoffmann, G. L.: Further Studies with Reference to Spirochætes Observed in Swine, Ztschr. f. Immunitätsf., 1914, xx, 347.
[100]King, W. E., and Drake, R. H.: Inoculation Experiment with Pure Culture of Spirochæta hyos, Jour. Infect. Dis., 1915, xvi, 54.
[101]Castellani, A.: On the Presence of Spirochætes in Two Cases of Ulcerated Parangi (yaws), Brit. Med. Jour., 1905, ii, 1280.
[102]Noguchi, H.: Cultural Studies on Mouth Spirochætes, Treponema microdentium and Macrodentium, 1912, xv, 81.
[103]Noguchi, H.: Treponema mucosum (n. sp.) a Mucin-producing Spirochæta from Pyorrhea Alveolaris, Grown in Pure Culture, Jour. Exper. Med., 1912, xvi, 194.
[104]Noguchi, H.: Cultivation of Treponema calligyrum (n. s.) from Condylomata of Man, Jour. Exper. Med., 1913, xvii, 89.
[105]MacNeal, W. J.: A Spirochæte Found in the Blood of a Wild Rat, Proc. Soc. Exper. Biol. and Med., 1907, iv, 125.
[106]Gaylord, H. R.: Spirochæte in Primary and Transplanted Carcinoma of the Breast in Mice, VIII Ann. Rep. of the Cancer Laboratory, 1908.
[107]Calkins, G. N.: A Spirochæte in Mouse Cancer, Spirochæta microgyrata, Jour. Infect. Dis., 1905, iv, 171.
[108]Borrel, A.: Infection vermineuse et spirochètes chez les souris cancéreuses, Compt. rend. Soc. de Biol., 1905, Series 12, i, 770.
[109]Tyzzer, E. E.: A Series of Spontaneous Tumors in Mice, Proc. Soc. Exper. Biol. and Med., 1907, iv, 85.
[110]Deetjen, H.: Spirochäten bei den Krebsgeschwülsten der Mäuse, München. med. Wchnschr., 1908, xxii, 1167.
[111]Mezinescu, D.: Sur une spirillose du rat, Compt. rend. Soc. de Biol., 1909, lxvi, 58.
[112]Smith, Th.: Grobe und feine Spirillen im Darm eines Schweines, Centralbl. f. Bakteriol., Abt. 1, 1894, xvi, 324.
[113]Dodd: A Disease of the Pig Due to a Spirochæta, Jour. comp. pathol. and therap., 1906, xix, 216.
[114]Futaki, Takaki, and Taniguchi: The Cause of Rat-bite Fever, Jour. Exper. Med., 1916, xxiii, 249.
[115]Weil, A.: Über eine eigenthümliche mit Milztumor Icterus und Nephritis einhergehende, acute Infectionskrankheit, Deutsch. Arch. f. klin. Med., 1886, xxxix, 209.
[116]Jaeger, H.: Die Aetologie des Infectiösen fieberhaften Icterus (Weil'sche Krankheit); etc., Z. f. Hyg. u. Infectionskrankh., 1892, xii, 525.
[117]Noguchi, H.: Additional Studies on the Presence of Spirochæta pallida in General Paralysis and Tabes Dorsalis, Jour. Cutan. Dis., 1913, xxxi, 543.
[118]Crohn, B. B.: Rat-bite Fever, Arch. Int. Med., 1915, xv, 1014.
[119]Noguchi, H.: Transmission of Treponema pallidum from the Brains of Paretics to the Rabbit, Jour. Am. Med. Assn., 1913, lxi, 85.
[120]Noguchi, H.: The Direct Cultivation of Treponema pallidum Pathogenic for the Monkey, Jour. Exper. Med., 1912, xv, 90.
[121]Noguchi, H.: A Method for Cultivating Treponema pallidum in Fluid Media, Jour. Exp. Med., 1912, xvi, 211.
[122]Bronfenbrenner, J., and Noguchi, H.: On the Resistance of Various Spirochætes in Cultures to the Action of Chemical and Physical Agents, Jour. Pharm. and Exp. Ther., 1912-13, iv, 333.
[123]Jacobs, W. A., and Heidelberger, M.: The Quarternary Salts of Hexamethylenetetramine, Jour. Biol. Chem., 1915, xx, 659, 685; Ibid., xxi, 103, 145, 403, 439, 455, 465.

[124]Jacobs, W. A.: The Problem of the Chemotherapy of Experimental Bacterial Infections, Jour. Exper. Med., 1916, xxiii, 563, 568.

[125]Jacobs, W. A.: The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. I. The Problem of the Chemotherapy of Experimental Bacterial Infections, Jour. Exper. Med., 1916, xxiii, 563.

[126]Jacobs, W. A., Heidelberger, M. and Amoss, H. L.: The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. II. The Relation Between Constitution and Bactericidal Action in the Substituted Benzylhexamethylenetetraminium Salts, Jour. Exper. Med., 1916, xxiii, 569.

[127]Jacobs, W. A., Heidelberger, M., and Bull, C. G.: The Bactericidal Properties of the Quarternary Salts of Hexamethylenetetramine. III. The Relation Between Constitution and Bactericidal Action in the Quarternary Salts Obtained from Halozenacetyl Compounds. Jour. Exper. Med., 1916, xxiii, 577.

[128]Ehrlich, P., and Hata, S.: Die experimentelle Chemotherapie der Spirillosen, Berlin, 1910.

[129]Marks, L. H.: Über einen arsenfesten Bakterienstamm, Ztschr. f. Immunitätsf., 1910, vi, 293.

(To be continued.)

THE EFFECT OF CERTAIN DRUGS UPON SKIN REACTIONS*

By John A. Kolmer, M.D., Samuel L. Immerman, M.D.,
Toitsu Matsunami, M.D., and Charles M. Montgomery, M.D.

THAT the oral administration of certain drugs may influence skin reactions to the extent of increasing their degree and severity or producing well marked papular or pustular reactions in the skins of persons who did not react to these injections in preliminary tests, has not been generally recognized until recently.

Amberg and Knox[1] found that the intravenous administration of neutral sodium ortho-iodoxybenzoate and sodium iodosobenzoate inhibited the development of reactions following the intracutaneous injection of serum in sensitized rabbits; sodium iodbenzoate and sodium benzoate in equal molecular concentrations had no inhibitory effect on the local reaction. The authors believed that the inhibitory influence of these drugs was attributable to their effect on oxidative processes. Opposite results have been reported more recently by Sherrick,[2] who found that the administration of potassium iodide either simultaneously, or shortly before or after the intracutaneous injection of luetin and agar, resulted in the production of pustular or nodular reactions in healthy nonsyphilitic persons. Kolmer, Matsunami, and Broadwell[3] confirmed these observations among healthy nonsyphilitic persons, persons sick with diseases other than syphilis and normal rabbits and guinea pigs. The oral administration of potassium iodide may cause the site of a former intracutaneous injection of luetin or agar to "light up" and present well defined inflammatory changes sometimes leading to pustulation as pointed out by Sherrick; we also found that a purer form of luetin prepared of washed spirochetes and free of all culture media, was influenced in a much lesser degree by potassium iodide.

Our studies have been continued to determine the influence of iodides, bromides, chlorides, ether and chloroform upon cutaneous, intracutaneous, and conjunctival tests; also upon the mechanism of the influence of drugs upon skin reactions.

The above mentioned drugs were selected on the basis of the investigations of Jobling and Petersen[4] who claim that the proteolytic ferments of the blood serum are held in check by antiferment in the nature of unsaturated fatty acid radicals and that the latter may be removed by saturation with iodine; also that other halogens and ether and chloroform may remove the antiferment from serum *in vitro* and increase tryptic activity.[5] It is possible that these drugs may influence the local skin reactions in this manner; that is, by increasing tryptic activity and the production of proteotoxins responsible for the lesion. Furthermore we were influenced in selecting the iodides and bromides in this study on the basis of abundant clinical evidence to the effect that their prolonged administration may result in the development of papular and pustular eruptions in the skin.

*From the McManes Laboratory of Experimental Pathology of the University of Pennsylvania, Philadelphia, Pa.

METHOD OF STUDY.

Three intracutaneous tests were conducted, (1) with a luetin prepared after the method of Noguchi and so diluted with sterile salt solution that the proper dose was contained in 0.1 c.c.; (2) with an emulsion of washed B. prodigiosis, resuspended in sterile salt solution so that each cubic centimeter contained about two billion bacilli, heated at 60° C. for one hour, preserved with 0.2 per cent tricresol and injected in dose of 0.1 c.c. This preparation has been designated "prodigiosin;" (3) with a 1:10,000 dilution of Koch's old tuberculin injected in

Fig. 1.—Dr. J. A. K.; intracutaneous and cutaneous reactions before the administration of potassium iodide.

amount of 0.1 c.c. Many of the reactions following the injection of this amount of tuberculin were quite severe, so that in a number of tests the amount injected was reduced to 0.05 c.c.

The cutaneous and conjunctival tests were conducted with undiluted and diluted Koch's old tuberculin.

Preliminary skin tests were made among adult persons who either volunteered or gave consent, and the reactions carefully measured and recorded forty-eight hours later. Wassermann reactions were made with the sera of all persons

receiving the intracutaneous luetin and prodigosin tests and with three different antigens; namely, a cholesterinized alcoholic extract of human heart, and alcoholic extract of syphilitic liver and an extract of acetone insoluble lipoids of beef heart.

The majority of persons were Wassermann negative with all antigens; in many persons, as laboratory assistants, medical students, and the authors, syphilis could be excluded with considerable certainty. A number of Wassermann positive and syphilitic persons and persons yielding positive tuberculin reactions

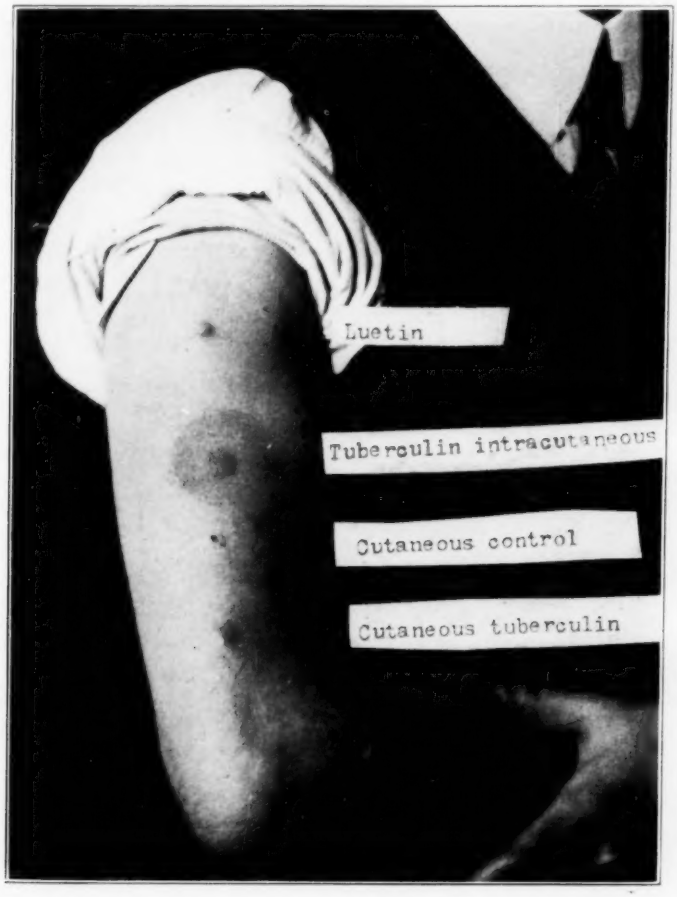


Fig. 2.—Dr. J. A. K.; intracutaneous and cutaneous reactions after the oral administration of 50 grains of potassium iodide.

were purposely included, in order to determine the influence of the drugs under study upon cutaneous and intracutaneous anaphylactic reactions.*

The drugs were administered by mouth in dose of 10 grains two or three times a day for a period of three days. On the fourth day the skin tests were repeated with the same materials and in exactly the same manner, the reactions being measured and recorded forty-eight hours later.

*We are indebted to Dr. J. Allen Jackson, Chief Resident Physician in the Department for the Insane, Philadelphia General Hospital, for cooperation and clinical facilities afforded us in the study of these cases.

The majority of persons were kept under observation for a period of ten days or longer to note the retarded effect, if any, of the drugs upon the first and second sets of skin tests.

As a general rule the luetin and prodigiosin tests were conducted together in all persons, the preliminary tests being made in the skin of the left arm and the second tests, in the skin of the right arm.

Both the luetin and prodigiosin produced reactions in the majority of persons in the preliminary tests. As a general rule the former yielded a slight

Fig. 3.—Mr. W. G.; intracutaneous and cutaneous reactions before the administration of potassium bromide.

reaction with an area of erythema measuring five millimeters or thereabouts and very slight edema; the prodigiosin reactions were more marked. In determining whether or not a drug had produced an effect we were influenced by the increased area of erythema, the greater infiltration, and frequently the development of a pustule. In each table we have given the size of each area of erythema and an interpretation of the results. Only decided differences in the degree of the reactions were accepted as indicating that the drug under study had increased the severity of the reactions.

RESULTS.

1. *The influence of potassium and sodium iodide; potassium and sodium bromide; potassium and sodium ammonium chloride; the protiodide of mercury and ether upon the luetin and prodigiosin reactions among Wassermann negative persons.*—The influence of these drugs administered in dose of 10 grains two or three times a day for a period of three days, upon the two intracutaneous tests are shown in Tables I to VI. The protiodide of mercury was included in the series by reason of its wide use in the treatment of syphilis and because the

Fig. 4.—Mr. W. G.; intracutaneous and cutaneous reactions after the administration of 60 grains of potassium bromide.

luetin reaction may be employed by the clinician while a patient is taking the drug. The results recorded in Table VII were observed after the administration of ¼ grain four times a day for a period of three days.

The influence of ether was studied by applying the tests before and after anesthesia for surgical operations. The amounts of ether used in the individual cases and the results observed are shown in Table VIII.

TABLE I

THE INFLUENCE OF POTASSIUM IODIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.6×0.5	1.0×0.8	0	0
2	0.5×0.5	1.0×0.8	0	0
3	0.6×0.6	0.8×0.5	0.8×0.8	1.0×1.0
4	0.6×0.4	1.0×0.5	1.0×0.5	1.5×1.0
5	0.7×0.7	1.0×0.8	0.7×0.4	2.5×2.0
6	0.8×0.7	1.5×1.0	1.5×1.5	2.0×1.5
7	0.6×0.6	1.2×1.0	1.5×1.5	1.5×1.5

Results: Luetin reactions influenced in Nos. 1, 2, 4 and 5; prodigiosin reactions influenced in Nos. 4, 5 and 6.

TABLE II

THE INFLUENCE OF SODIUM IODIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	1.0×1.0	1.0×1.0	1.2×0.8	2.0×1.5
2	0.6×0.8	1.0×1.0	0.8×0.6	1.0×0.8
3	0.6×0.6	1.0×0.8	0.6×0.8	1.0×0.8
4	0.2×0.2	0.2×0.2	0.6×0.4	2.5×2.0
5	0.5×0.5	0.5×0.5	0.6×0.6	0.8×0.6
6	0.8×0.8	1.2×1.0	1.0×1.0	1.0×1.0
7	0.4×0.4	0.6×0.5	0.8×0.6	1.5×1.0
8	0.5×0.5	0.8×1.0	1.5×1.0	1.2×1.5
9	0.8×0.5	0.7×1.2	1.2×0.6	1.2×1.5

Results: Luetin reactions influenced in Nos. 2, 3, 6 and 8; prodigiosin reactions influenced in Nos. 1, 2, 3, 4 and 7.

TABLE III

THE INFLUENCE OF POTASSIUM BROMIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.6×0.3	0.6×0.5	0.6×0.4	1.0×0.8
2	0.9×0.8	1.0×1.0	0.9×0.8	2.0×2.0
3	1.0×0.7	1.0×0.5	0.8×0.5	2.5×2.0
4	0.7×0.6	0.5×0.5	0.6×0.6	2.0×2.0
5	0.8×0.8	0.6×0.6	0.9×0.6	1.0×1.0
6	0.2×0.2	0.6×0.6	0	0
7	0.5×0.5	0.6×0.8	0	0
8	0.1×0.2	0.8×0.6	0.6×0.6	1.0×1.5
9	0.6×0.5	0.6×0.6	0.5×0.5	0.5×0.5

Results: Luetin reactions influenced in Nos. 6 and 8; prodigiosin reactions influenced in Nos. 1, 2, 3, 4 and 8.

TABLE IV

THE INFLUENCE OF SODIUM BROMIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.3×0.3	1.0×1.5	0.4×0.8	2.0×2.0
2	0.5×0.4	0.5×0.5	0.5×0.3	1.0×0.8
3	0.9×0.6	0.8×0.5	0.8×0.5	2.0×1.5
4	0.4×0.4	0.8×0.5	0.4×0.4	2.0×2.0
5	0.8×0.5	0.8×0.5	1.0×1.0	2.0×1.5
6	0.4×0.2	0.5×0.3	0.7×0.7	0.9×0.8
7	0.4×0.3	0.8×0.8	0.8×0.6	2.0×2.0

Results: Luetin reactions influenced in Nos. 1 and 7; prodigiosin reactions influenced in Nos. 1, 2, 3, 4, 5 and 7.

TABLE V

INFLUENCE OF POTASSIUM CHLORIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.1×0.1	0.5×0.5	0.2×0.2	1.0×1.0
2	1.0×0.4	0.5×0.5	0.6×0.5	1.0×1.5
3	0.6×0.4	0.6×0.5	1.6×0.6	1.5×1.0
4	0.5×0.5	0.5×0.5	1.2×0.2	1.0×1.0
5	0.5×0.5	0.5×0.5	1.1×1.0	1.0×1.0
6	0.6×0.6	0.6×0.5	0.6×0.4	1.0×1.0
7	0.5×0.6	0.5×0.5	0	0
8	0.5×0.5	0.6×0.6	0	0
9	0.5×0.5	0.5×0.5	1.0×0.4	1.0×1.4

Results: Luetin reaction influenced in No. 1; prodigiosin reactions influenced in Nos. 1, 2 and 4.

TABLE VI

INFLUENCE OF AMMONIUM CHLORIDE UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.5×0.5	0.5×0.5	0.6×0.6	1.0×1.0
2	0.6×0.5	0.5×0.5	0.6×0.5	0.6×0.6
3	0.8×0.5	0.5×0.5	0.4×0.4	1.0×1.0
4	0.6×0.5	0.5×0.4	0.6×0.6	0.7×0.5
5	0.7×0.6	0.5×0.5	1.6×0.6	1.0×0.6
6	0.8×0.8	0.5×0.5	1.0×0.6	1.0×1.0
7	0.5×0.4	0.6×0.8	0.6×0.5	1.0×1.0
8	0.5×0.5	0.5×0.5	0.4×0.4	1.0×1.0
9	0.5×0.5	0.4×0.4	1.2×1.0	1.5×1.8
10	0.8×0.8	0.8×0.8	2.0×3.0	2.5×3.0

Results: Luetin reactions influenced in none; prodigiosin reactions influenced in Nos. 1, 3, 7 and 8.

TABLE VII

INFLUENCE OF PROTIODIDE OF MERCURY UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

No.	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
	Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	0.5×0.8	0.5×0.5	0.6×0.5	0.6×0.8
2	0.1×0.2	0.4×0.8	1.0×1.0	3.0×3.0
3	0.8×0.4	0.5×0.5	0.6×0.4	1.0×1.0
4	0.8×0.5	0.5×0.8	0.8×0.8	0.8×0.8
5	0.4×0.2	0.3×0.3	0.6×0.3	0.5×0.5
6	0.6×0.8	0.6×0.6	0.5×0.5	0.5×0.5

Results: Luetin reaction influenced in No. 2; prodigiosin reactions influenced in Nos. 2 and 3.

TABLE VIII

THE EFFECT OF ETHER ANESTHESIA UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS

NO.	AMOUNT OF ETHER USED	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
		Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	12 ounces	0.5×0.5	0.6×0.6	1.2×1.0	1.0×1.0
2	10 ounces	0.7×0.7	0.6×0.6	1.5×1.0	1.2×1.0
3	8 ounces	0.7×0.7	0.9×0.8	1.0×1.0	1.3×1.0
4*	15 ounces	0.8×0.8	0.8×0.8	1.0×0.8	1.0×1.0
5*	8 ounces	1.3×1.0	1.0×0.7	0.6×0.5	0.6×0.4
6*	11 ounces	0.5×0.3	0.5×0.5	1.0×0.6	0.7×0.7
7*	16 ounces	0.5×0.5	0.7×0.7	0.6×0.4	0.6×0.5

* Patients received morphine sulphate, gr. 1/4 and atropine sulphate, gr. 1/100, before ether was administered.

Results: Luetin reactions not influenced; prodigiosin reactions not influenced.

A summary of the results is shown in Table IX, which gives the percentage of reactions apparently influenced; that is, rendered more extensive (wider erythema and greater infiltration), by the various drugs.

TABLE IX

THE EFFECT OF DRUGS UPON THE INTRACUTANEOUS LUETIN AND PRODIGIOSIN REACTIONS AMONG WASSERMANN NEGATIVE PERSONS

DRUGS	TOTAL	PERCENTAGE OF REACTIONS INFLUENCED BY THE DRUG	
		LUETIN	PRODIGIOSIN
Potassium iodide	7	71	60
Sodium iodide	9	44	55
Protiodide of mercury	6	16	33
Potassium bromide	9	22	55
Sodium bromide	7	28	85
Potassium chloride	9	10	33
Ammonium chloride	10	0	40
Ether	7	0	0
Nitrous oxide	3	0	0

As shown in the tables, certain of the drugs used in this study were found to influence the tests by increasing the inflammatory reactions.

a. The prodigiosin reactions were generally more extensive than the luetin reactions and were more readily influenced by certain of the drugs. As previously stated, we have found that the reactions produced by the injection of 0.4 per cent agar-agar were more readily influenced by potassium iodide than the luetin reaction; also that the reaction produced by luetin prepared in the usual manner was more readily influenced than that prepared of washed spirochetes and free of the culture medium (ascites agar). In other words substances most likely to excite inflammatory reactions in the skin appear to be most readily influenced by the iodides and to some extent by bromides.

b. The iodides and particularly the potassium salt, seemed to exert more influence than the other compounds used in this study.

c. The bromides were found to influence these reactions and particularly the prodigiosin reactions, but to a lesser degree than the iodides.

d. The chlorides were found to be practically without effect upon the luetin reactions, although a number of the prodigiosin reactions appeared to be influenced.

e. Nitrous oxide and ether by inhalation had no appreciable influence upon the reactions.

2. *The effect of sodium iodide; potassium and sodium bromide; potassium and ammonium chloride; ether and chloroform upon the intracutaneous luetin and agar reactions in rabbits.*

Preliminary intracutaneous tests (abdominal skin) with luetin and a 0.4 per cent sterile solution of agar-agar, were made in a series of rabbits and the reactions recorded forty-eight hours later.

The drugs were dissolved in water and administered once daily by means of the stomach tube in dose of one grain per sixty kilograms of body weight.

The skin tests were repeated after the third dose and again after six additional doses.

The influence of ether was studied by anesthetizing rabbits for one hour and repeating the luetin and agar tests two hours later; the influence of chloroform was studied in the same manner except that the period of anesthesia was thirty minutes.

The results were indefinite and difficult to read and interpret, due mainly to the difficulty of making an injection into the abdominal skin of a rabbit. As a general rule the reactions to agar were more marked than those excited by the luetin.

In no instance were the reactions influenced by three doses of the drugs. In two animals the reactions were intensified after additional doses of potassium iodide, but the other drugs used in this study were without appreciable effect. The agar reactions were influenced by potassium iodide to a greater extent than the luetin reactions.

Conjunctival tuberculin tests among a series of nontuberculous animals before and after the administration of iodides, bromides and chlorides were negative throughout.

3. *The influence of potassium iodide, potassium bromide and potassium chloride upon cutaneous and intracutaneous tuberculin tests.*—Of 47 adults upon whom the cutaneous tuberculin test of von Pirquet was conducted, six did not show any reaction. Potassium iodide was then administered, the total amount of drug taken varying from 20 to 160 grains, and the tuberculin tests repeated. One person reacted quite distinctly in the second test with "lighting up" of the preliminary test several days later, but we are unable to state whether these effects are to be ascribed to the influence of the potassium iodide or to a delayed tuberculin reaction. A second person presented a mild reaction in the second test.

In a second group of adults the cutaneous and intracutaneous tests were conducted simultaneously on the left arm. All of the reactions were positive and were carefully measured forty-eight hours later; the following drugs were then administered in doses of 10 grains, twice daily for a period of three days: potassium iodide, potassium bromide and potassium chloride. Both tests were then repeated on the left arm and the reactions measured and recorded forty-eight hours later. The results are shown in Table X.

TABLE X

THE EFFECT OF DRUGS UPON THE CUTANEOUS AND INTRACUTANEOUS TUBERCULIN REACTIONS

NAME	DRUG	CUTANEOUS TUBERCULIN		INTRACUTANEOUS TUBERCULIN	
		Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
Dr. E. S...	Potass. iodide....	1.0×1.0	1.5×1.5	3.0×4.0	3.0×4.0
Dr. J. A. K.	Potass. iodide....	1.6×1.5	3.0×3.0	1.7×1.6	4.1×4.0
Dr. T. M..	Potass. brom.....	1.0×1.5	1.0×1.5	2.0×2.0	2.0×1.5
Mr. W. G..	Potass. brom.....	0.5×0.5	0.8×1.0	2.5×2.5	2.0×2.5
Dr. S. I...	Potass. brom.....	1.0×1.0	2.3×2.0	4.0×4.0	5.0×5.5
Dr. B. M..	Potass. chloride...	1.0×1.0	1.0×1.0	4.0×4.0	4.5×4.5
Mr. C. H.	Potass. chloride...	0.5×0.6	0.8×0.6	2.8×2.5	3.5×3.0

TABLE XI

EFFECT OF DRUGS UPON THE LUETIN AND PRODIGIOSIN INTRACUTANEOUS REACTIONS AMONG WASSERMANN POSITIVE PERSONS

NO.	WASSERMANN REACTION	DRUG	LUETIN REACTIONS		PRODIGIOSIN REACTIONS	
			Before (cm.)	After (cm.)	Before (cm.)	After (cm.)
1	Strongly Positive.....	Potass. iodide.....	1.0×1.2	1.5×1.8	0.7×0.4	0.8×0.5
2	Strongly Positive.....	Sod. iodide.....	1.0×0.6	1.5×1.5	0.8×1.0	2.0×2.0
3	Weakly Positive.....	Protiodid. mercury..	1.0×0.8	1.0×1.0	1.0×1.0	3.0×2.5
4	Strongly Positive.....	Potass. brom.....	0.2×0.4	0.3×0.4	0.5×0.4	1.0×1.0
5	Weakly Positive.....	Sod. brom.....	0.8×0.5	0.6×0.5	1.0×1.0	2.0×1.5
6	Moderately Positive...	Sod. brom.....	1.3×1.2	1.0×1.0	1.0×0.6	1.0×1.5
7	Weakly Positive.....	Potass. chlor.....	1.0×1.0	1.0×1.0	1.0×0.5	1.0×0.8
8	Strongly Positive.....	Ether *.....	1.0×1.0	1.0×0.8	0.9×0.9	1.0×1.0

* 10 ounces.

Results: Luetin reactions regarded as positive in Nos. 1, 2, 3, 6, 7, 8 and influenced in Nos. 1 and 2; prodigiosin reactions influenced in Nos. 2, 3, 4 and 5.

Potassium iodide was found to influence both the cutaneous and intracutaneous reactions of two persons as evidenced by wider inflammatory areolæ the greater edema (Dr. E. S. and Dr. J. A. K.).

Potassium bromide produced a similar influence upon the reactions of one of three persons (Dr. S. I.).

Potassium chloride did not produce any appreciable influence upon the cutaneous reactions of two persons, but increased very slightly the intracutaneous reaction of one of these persons (Mr. C. H.).

It appears that these drugs and particularly potassium iodide, may increase the inflammatory phenomena of an anaphylactic reaction although in these tuberculin reactions the second reactions may have been influenced by the previous tests. Among persons in the tertiary stage of syphilis, presenting positive Wassermann reactions and positive luetin reactions in most instances, potassium iodide appeared to increase the degree of reaction to luetin, while the prodigiosin reactions were influenced to a greater degree (Table XI).

MECHANISM OF THE ACTION OF DRUGS UPON SKIN REACTIONS.

Skin reactions may be divided into three varieties, (1) the true anaphylactic reaction due to the interaction in the skin of specific protein antigen and specific antibody; (2) the pseudo or nonspecific protein reaction due to the interaction in the skin of general protein substances and nonspecific proteolysins; (3) the traumatic reaction consequent to the operation; or to the irritant qualities of such substances as preformed bacterial toxins and various preservatives as phenol and tricresol contained in the injected material. One of us (J. A. K.) has discussed elsewhere our present knowledge bearing upon the mechanism and clinical significance of these reactions.[6] Certain drugs, and notably the iodides and to a lesser extent the bromides, may influence skin tests by increasing the severity of the reactions. We are of the opinion that this influence is exerted upon the nonspecific and traumatic reactions rather than upon the specific anaphylactic reaction, although definite proof of this assertion is not at hand. Intracutaneous tests are more readily influenced than cutaneous tests; as a rule the reactions produced by the injection of material of irritant properties are most readily influenced; as for example, the injection of agar-agar or ordinary luetin as compared with a luetin of washed spirochetes alone and free of culture medium. Iodides apparently increase the purely inflammatory and suppurative phases of skin reactions.

One of us (J. A. K.) has also tested the influence of potassium iodide upon the Schick toxin reaction in a few persons; the drug apparently increased the severity of the reactions among persons reacting positively and produced mild reactions among a few persons who possessed an antitoxic immunity of sufficient degree to yield negative toxin tests in the preliminary trials.

Definite knowledge of the mechanism of skin reactions is lacking, but there appears to be sufficient experimental data at hand to indicate that the nonspecific and traumatic skin reactions implicate the proteolytic ferments of fixed tissue cells, leucocytes, and serum, which produce from the injected protein and probably from the protein of the person's own serum a soluble toxic substance responsible in part for the reaction in the skin of erythema, edema, and leucocytic infiltration. If this is correct it would appear probable that the iodides, bromides and to a lesser extent the chlorides, are capable of increasing tryptic activity

by removal of antiferment, after the hypothesis of Jobling and Petersen previously mentioned.

As many of the reactions influenced by potassium iodide become pustular with extensive infiltration of the tissues with polymorphonuclear leucocytes, it is possible that the iodide may influence the leucocytes and facilitate the phagocytosis of the injected foreign material producing a heightened inflammatory reaction analogous to the focal reaction not infrequently following the administration of a bacterial vaccine in adequate dosage.

Daily total and differential leucocyte counts of the blood of two persons taking potassium iodide and two others taking potassium bromide until a total of 170 grains has been swallowed, did not show any material influence upon either the total number or variety of leucocytes in the peripheral blood, as compared with a series of preliminary counts before the administration of the drugs. Similar negative results were observed with a series of rabbits receiving the drugs by means of a stomach tube.

The sera of persons and rabbits receiving potassium iodide and potassium bromide appears to have an appreciable increased power for facilitating phagocytosis. As shown in Tables XII and XIII, the sera of persons taking potassium iodide and potassium bromide showed higher phagocytic and opsonic indices for B. prodigiosis than the same sera in the preliminary tests before the administration of the drugs; this was particularly apparent with the sera of persons taking

TABLE XII

INFLUENCE OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND POTASSIUM BROMIDE UPON PHAGOCYTOSIS OF B. PRODIGIOSIS *

NAME	DRUG	PHAGOCYTIC INDICES **							
		1st Prelim.	2nd Prelim.	3rd Prelim.	4th Prelim.	After 60 Grains	After 90 Grains	After 140 Grains	After 170 Grains
C. E.	Potass. iodide.....	0.4	0.8	1.0	0.9	1.9	2.6	1.7	1.9
G. H.	Potass. iodide.....	0.4	0.4	1.3	1.6	3.4	1.7	2.0	2.4
A. B.	Potass. bromide.....	0.3	1.0	2.4	1.1	1.9	1.6	0.9	...
S. H.	Potass. bromide.....	0.2	0.6	1.0	0.9	1.9	3.6	1.7	...

* Tests conducted with the leucocytes of a normal person and not with the patient's own leucocytes.
** Phagocytic indices—the average number of bacilli ingested per leucocyte.

TABLE XIII

INFLUENCE OF THE ORAL ADMINISTRATION OF POTASSIUM IODIDE AND POTASSIUM BROMIDE UPON THE OPSONIC INDEX (B. PRODIGIOSIS) *

NAME	DRUG	AVERAGE PHAGOCYTIC INDEX	OPSONIC INDICES **			
			After 60 Grains	After 90 Grains	After 140 Grains	After 170 Grains
C. E.	Potass. iodide.....	0.7	2.8	3.5	2.4	2.8
G. H.	Potass. iodide.....	0.9	4.0	2.0	2.2	2.7
A. B.	Potass. bromide.....	1.2	1.5	1.3	0	....
S. H.	Potass. bromide.....	0.7	2.8	5.0	2.4	....

* Tests conducted with the leucocytes of a normal person and not with the patient's own leucocytes.
** Opsonic indices obtained by dividing the patient's phagocytic index by the average phagocytic index obtained with his serum before the administration of the drugs (see Table XII).

TABLE XIV

INFLUENCE OF ORAL ADMINISTRATION OF POTASSIUM IODIDE AND PHAGOCYTOSIS OF B. PRODIGIOSIS *

RABBIT NO.	PHAGOCYTIC INDICES **								
	1st Prelim.	2nd Prelim.	3rd Prelim.	After 1st Dose	After 2nd Dose	After 3rd Dose	After 4th Dose	After 5th Dose	After 6th Dose
1.....	0.4	1.3	0.4	0.7	0.5	1.5	2.6	5.6	6.7
2.....	0.5	0.4	1.1	0.7	2.5				
3.....	0.3	3.3	1.2	2.8	3.2	3.4	5.3	9.4	10.7
4.....	0.7	1.2	1.9	1.2	1.1	1.6	3.0	4.9	6.2

* Tests conducted with the leucocytes of a normal rabbit.

TABLE XV

INFLUENCE OF ORAL ADMINISTRATION OF POTASSIUM IODIDE UPON THE OPSONIC INDEX (B. PRODIGIOSIS) *

RABBIT NO.	AVERAGE PHAGOCYTIC INDEX	OPSONIC INDICES					
		After 1st Dose	After 2nd Dose	After 3rd Dose	After 4th Dose	After 5th Dose	After 6th Dose
1.....	0.7	1.0	0.7	2.0	3.7	8.0	9.5
2.....	0.7	1.0	3.5				
3.....	1.6	1.8	2.0	2.1	3.3	5.8	6.7
4.....	1.3	0.9	0.8	1.2	2.3	3.7	4.8

* Conducted with the leucocytes of a normal rabbit.

potassium iodide. Even more marked results were observed with the sera of rabbits receiving potassium iodide by means of the stomach tube (Tables XIV and XV).

Phagocytic experiments *in vitro* consisting in exposing emulsions of B. prodigiosis and staphylococcus aureus to varying dilutions of the drugs in normal salt solution under study for an hour at 37° C., followed by the addition of an emulsion of the mixed leucocytes of normal rabbits secured by aleuronant irritation and from the peripheral blood and reincubation for half to an hour, were generally negative. As shown in Table XVI, the 1:4000 dilution of potassium iodide and potassium bromide appeared to raise the phagocytic powers of the leucocytes, while the 1:4000 dilution of potassium chloride exerted a similar, but very slight influence. A similar influence on the part of the drugs upon staphylococcus aureus was not apparent (Table XVII). Occasionally well marked phagocytosis was observed but not higher than shown occasionally in the series of controls.

It would appear, therefore, that the oral administration of potassium iodide and to a lesser extent of potassium bromide, may increase the phagocytic power of the blood serum for B. prodigiosis and this may have some influence upon skin reactions by increasing leucocytic infiltration about the injected mass. Whether these drugs have a similar influence upon other microorganisms or upon the phenomena of infection and resistance in general would appear worthy of further investigation.

TABLE XVI

INFLUENCE OF DRUGS UPON THE PHAGOCYTOSIS OF B. PRODIGIOSIS IN VITRO *

DILUTIONS OF DRUGS **	PHAGOCYTIC INDICES		
	Potass. Iodide	Potass. Bromide	Potass. Chloride
1:200	1.2	1.1	2.2
1:1000	1.1	1.5	2.0
1:4000	2.5	3.5	2.9
1:10,000	0.9	1.4	1.8
Control ***	1.1	2.1	1.5

* The leucocytes of a normal rabbit used.
** In sterile normal salt solution (0.85 per cent).
*** In sterile normal salt solution (0.85 per cent).

TABLE XVII

INFLUENCE OF DRUGS UPON THE PHAGOCYTOSIS OF STAPHYLOCOCCUS AUREUS IN VITRO *

DILUTIONS OF DRUG * * *	PHAGOCYTIC INDICES **					
	Potass. Iodide	Sodium Iodide	Potass. Bromide	Sodium Bromide	Potass. Chloride	Ammon. Chloride
1:100	0.9	0.7	0.9	1.0	1.2	0.4
1:500	0.8	0.9	0.5	1.3	0.8	0.5
1:1000	1.1	0.9	0.9	1.2	0.8	0.8
1:5000	0.9	0.5	1.0	1.4	0.8	0.8
1:10,000	0.9	0.5	1.4	1.5	1.3	0.5
1:20,000	1.3	0.8	0.7	0.9	1.2	0.9
1:40,000	0.3	1.2	0.6	1.3	0.8	0.6
1:100,000	1.1	0.5	0.7	1.0	0.8	0.5

* Staphylococcus aureus recently cultivated from the pus of a furuncle. The leucocytes of normal rabbits employed.
** The controls (set up with sterile normal salt solution) showed a spontaneous phagocytosis varying from 2.0 to 0.1 and averaging 0.5 per leucocyte.
*** In sterile normal salt solution (0.85 per cent).

SUMMARY.

1. The iodides and particularly potassium iodide were found to influence the luetin and prodigiosis intracutaneous tests to a marked extent. Normal non-syphilitic persons, reacting negatively in the luetin test, may show marked reactions when tested after the oral administration of sixty or more grains of potassium iodide.

2. The bromides of potassium and sodium in the same dosage were found to have a similar but less marked influence.

3. The chlorides of potassium and ammonium in the same dosage were found to influence the prodigiosin reactions but not the luetin reactions except to a very slight extent.

4. The administration of the protiodide of mercury influenced the luetin reaction to some extent.

5. It is probable that the administration of larger doses of these drugs would exert a more marked influence upon skin reactions.

6. Ether and chloroform anesthesia did not appear to influence skin reactions.

7. Substances most likely to excite inflammatory reactions in the skin ap-

pear to be most readily influenced by the iodides and to some extent by bromides; intracutaneous tests with agar-agar, prodigiosin and ordinary luetin were more readily influenced by these drugs than the reactions following the injection of a luetin of washed spirochetes and free of culture medium.

8. Cutaneous tests are not as readily influenced by these drugs as intracutaneous tests.

9. Conjunctival tests among normal rabbits to tuberculin were apparently not influenced.

10. Cutaneous and intracutaneous reactions to tuberculin among persons reacting positively to both, appear to be rendered more extensive by potassium iodide and to a lesser extent by potassium bromide.

11. Anaphylactic reactions to luetin in syphilitic persons appear to be rendered more extensive by potassium iodide and potassium bromide.

12. The oral administration of potassium iodide and to a lesser extent of potassium bromide, increased the phagocytic power of the blood serum for B. prodigiosis; the increased severity of skin reactions in persons taking these drugs may be due to heightened leucocytic infiltration and phagocytosis about the injected material or to an increase of tryptic activity through the saturation of fatty acid radicals according to the hypothesis of Jobling and Petersen.

13. Physicians should very carefully rule out the possible influence of these drugs before conducting skin reactions.

14. It is probable that these drugs have influenced the luetin reactions as clinically applied and have been responsible in part for the divergent results observed and reported.

BIBLIOGRAPHY.

[1]Amberg, S., and Knox, J. H. M.: Influence of Sodium Iodoxybenzoate on Reactions of Inflammatory Character, Jour. Am. Med. Assn., 1912, lix, 1598-1599.

[2]Sherrick, J. W.: The Effect of Potassium Iodide on the Luetin Reaction, Jour. Am. Med. Assn., 1915, lxv, 404-405.

[3]Kolmer, J. A., Matsunami, T., and Broadwell, S.: The Effect of Potassium Iodide on the Luetin Reaction, Jour. Am. Med. Assn., 1916, lxvii, 718-719.

[4]Jobling, J. W., and Petersen, W.: The Therapeutic Effect of Iodine, Jour. Am. Med. Assn., 1914, lxiii, 1930-1931.

[5]Jobling, J. W., and Petersen, W.: Serotoxin Studies on Ferment Action, XIV, Jour. Exper. Med., Lancaster, 1914, xix, 480-500.

[6]Kolmer, J. A.: The Mechanism and Clinical Significance of Anaphylactic and Pseudo-anaphylactic Skin Reactions, Bull. Johns Hopkins Hosp. (in press).

THE DISTRIBUTION OF THE BLOOD SUGAR AS REGARDS CORPUSCLES, PLASMA, AND WHOLE BLOOD IN HEALTH AND DISEASE IN MAN*

By R. B. H. Gradwohl, M.D., and A. J. Blaivas, St. Louis, Mo.

IT occurred to the writers to determine according to the newer blood chemical methods the exact distribution of the blood sugar in man as regards the corpuscles, plasma, and whole blood. The data which we have accumulated are based upon a number of analyses of blood obtained in the routine way by venipuncture, the blood in all cases being received into potassium oxalate (dried), and the sample taken in the morning before breakfast. The method used was that of Benedict and Lewis, as modified by Myers and Bailey. It is interesting to note that so far as we are able to learn in a search of the literature, no such data have hitherto been obtained by using these methods.

Possibly the most important research along this line is that of Tachau[1] who reported his data on the distribution of blood sugar in blood corpuscles and blood serum. Prior to this publication, Lèpine,[2] Michaelis and Rona,[3] and Hollinger[4] found in the blood serum and corpuscles of man and other animals different amounts of blood sugar. Since then, others have worked along similar lines, notably Rona and Döblin,[5] E. Frank,[6] Lyttkens and Sandgreen,[7] Hoeber,[8] Schirokauer,[9] and others. There are great discrepancies in these results, possibly due to the fact that some work was carried out with human blood and other work with animal blood. Since the appearance of Masing's[10] and Loeb's[11] articles, we know that the sugar content of blood in different animals and in man gives different figures, and also that the variations in the blood sugar in man and in animals quite close to him are different from a metabolic standpoint. It must, however, be noted that the observations of most of these investigators have not been made with respect to an estimation of the normal blood sugar content under ordinary conditions, most of the human data having been based upon a computation after the ingestion of large amounts of carbohydrates and most of the animal data having been procured after the animals were narcotized and tied up for a long time. Michaelis and Rona[12] and E. Frank[13] showed that in the presence of a hyperglycemia due to the ingestion of a large amount of carbohydrates, the blood sugar content of serum is increased more than that of the whole blood or the corpuscles. As for the results in man, Hollinger found that the amount of sugar was the same in whole blood and in plasma. E. Frank, in a number of pathological cases, found more sugar in the serum. Schirokauer, as a rule, in fasting persons, found a higher percentage of blood sugar in plasma than in whole blood or corpuscles. In alimentary hyperglycemia the difference between the whole blood and the plasma was quite marked in a number of cases examined. It was noted that one hour after the ingestion of the dose of carbohydrate that caused the alimentary hyperglycemia, the balance between the two was adjusted so that there was practically no more difference between the blood

*From the Chemical Laboratory of the Pasteur Institute of St. Louis.

sugar content in carbohydrate-fed persons than that seen in fasting persons. The blood sugar in the corpuscles of alimentary hyperglycemics ran high; in but few cases did the concentration of sugar in the corpuscles remain low; and in one case, when the blood sugar concentration in the whole blood went up, the blood sugar in the corpuscles went down.

As for the different results seen in the case of man, Hollinger found the same amounts of sugar in plasma and in whole blood. E. Frank, in pathological cases, found an increase in the sugar in the serum over the corpuscles, while Schirokauer found great differences between the whole blood and the serum. Tachau worked this matter out on fasting persons. At the same time, Rolly and Oppermann[14] published their figures on fasting persons. Tachau reported both pathological cases and also normal people after carbohydrate ingestion. Owing to the fact that very large quantities of blood were needed with this technic Tachau could not use the same person's blood more than once. His technic was as follows: Blood obtained by venipuncture was received in sodium fluoride, 40 c.c. in quantity, centrifuged in a high power electric centrifuge for fifteen minutes. The blood volume was taken with a Boenning tube. The whole blood and corpuscles were treated according to Schenk's method to precipitate the protein material, and blood sugar estimation made with the Knapp solution as previously reported by Tachau.[15] A point to be taken into consideration in this work is the suggestion made by Lèpine[16] that there is "free" and "bound" sugar in the blood; i.e., that shortly after standing, within fifteen minutes, in fact, some of the "bound" sugar is liberated and becomes "free" sugar, going over from corpuscles to plasma, remaining of course in the plasma. Tachau covered this question in his investigations. A part of the blood was placed directly in water and 2 per cent hydrochloric acid as described in his previous technic; another part was received into the sodium fluoride and allowed to stand one hour in the laboratory. The blood volume was determined by weighing. Table I of Tachau's experiments gives the results on this question.

TABLE I

CASE NO.		PER CENT.	PER CENT AFTER 1 HR.
1.	Potator (after 100 gm. grape sugar)	0.113	0.111
2.	Liver cirrhosis " " " " "	0.142	0.145
3.	" " " " " " "	0.143	0.142
		0.148	0.148
4.	Erysipelas " " " " "	0.185	0.183
5.	Diabetes (fasting patient)	0.258	0.240
6.	Liver cirrhosis, (after 100 gm. grape sugar)	0.169	0.177
7.	Gout " " " " "	0.108	0.112
			0.114
8.	Normal, " " " " "	0.086	0.095
9.	Diabetic, fasting " " " " "	0.113	0.125

It will be noted that in the first four cases, the difference between the blood sugar content when first withdrawn and after one hour standing is so slight as to be negligible. In case 5, a fasting diabetic, blood sugar dropped from 0.258 to 0.240 per cent; here perhaps the sodium fluoride caused glycolysis.

In the last four cases, the blood sugar rose after one hour. It can be seen therefore that there is some slight rise, but this is not a constant or important factor.

In Table II of Tachau's figures are seen the results of investigations on fasting people.

TABLE II

INVESTIGATIONS ON FASTING PERSONS

Case No.	Diagnosis	SUGAR CONTENT							
		Whole Blood %	Plasma %	Difference Between Plasma and Whole Blood %	Quotient Plasma / Whole Blood	Blood Volume %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma / Corpuscles
1	Pregnancy	0.0765 0.0740	0.087	0.012	1.16	36	0.058 0.051	0.029 0.036	1.5 1.7
2	Arteriosclerosis	0.098	0.105	0.007	0.07	30	0.082	0.023	1.3
3	Uremia	0.105 0.104	0.110	0.005	1.05	34	0.095	0.015	1.2
4	Nephritis	0.111	0.126	0.015	1.14	45	0.093	0.033	1.4
5	Diabetes	0.129	0.138	0.009	1.07	50	0.120	0.018	1.2
6	"	0.150	0.173	0.023	1.15	47	0.125	0.048	1.4
7	"	0.153 0.156	0.168	0.014	1.09	....	....	....	....
8	"	0.165	0.165	0	1.00	27	0.165	0	1.0
9	"	0.183	0.185	0.002	1.01	42	0.181	0.004	1.0
10	"	0.243	0.246	0.003	1.01	....	....	....	....
11	"	0.258	0.265	0.007	1.03	....	....	....	....
12	"	0.301 0.306	0.323	0.022 0.017	1.07 1.06	21	0.219 0.243	0.104 0.080	1.5 1.3

With the exception of case 8, in which all the figures tallied, the sugar concentration was higher in plasma than in whole blood. The average difference was 0.01 per cent. The average quotient was 1.07. As for the volume, the difference between the plasma and the whole blood was greater in ratio to the blood volume; i.e., the greater the difference, the smaller the blood volume. The greatest difference between the plasma and the corpuscles was 0.104 per cent, an average of 0.030 per cent. The quotient average of plasma over corpuscles is 1.3. There seemed, therefore, no more difference between the blood sugar concentration in whole blood, plasma, and corpuscles in individuals with a high or low blood sugar.

Table III by Tachau illustrates the data on blood after the administration of carbohydrates.

The differences between the sugar in the plasma and in the whole blood or corpuscles are greater the higher the hyperglycemia, as opposed to the condition existing in fasting persons. The greatest difference occurred in case 11, 0.144 per cent, in a diabetic, the quotient of whole blood over plasma being 1.4. The quotient of plasma over corpuscles was in most cases as much as 2.0. In twelve of these cases where the patients were given carbohydrates followed by a blood test, the quotient of plasma over whole blood was five times higher than in fasting persons (Table II). In the cases where the examinations were

TABLE III

EXAMINATIONS AFTER INSTITUTION OF CARBOHYDRATES

Case No.	Diagnosis	Remarks	SUGAR CONTENT							
			Whole Blood %	Plasma %	Difference Between Whole Blood and Plasma %	Quotient Plasma / Whole Blood	Blood Volume %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma / Corpuscles
1	Healthy	1 hr. After 100 Gm. Grape Sugar	0.090	0.112	0.022	1.25	.....	.....	.....	.....
2	Heart Insufficiency	"	0.093	0.118	0.022	1.29	40	0.053	0.055	2.3
		"	0.096	0.120	0.027	1.23	40	0.063	0.063	1.8
3	Gout	"	0.112 0.114	0.129	0.016	1.15	.....	.....	.....	.....
4	Nephritis	"	0.142	0.155	0.013	1.09	45	0.127	0.028	1.2
5	Liver Cirrhosis	"	0.143	0.153	0.005	1.03	.....	.....	.....	.....
6	Uremia	"	0.148	0.155	0.002	1.08	.....	.....	.....	.....
7	Liver Cirrhosis	"	0.147	0.160	0.013	1.09	.....	.....	.....	.....
8	Erysipelas	"	0.169	0.213	0.044	1.26	39	0.100	0.113	2.1
9	Lead Poisoning	"	0.182 0.185	0.207	0.024	1.13	.....	.....	.....	.....
10	Diabetes	1 hr. After 50 Gm. White Bread	0.213	0.237	0.024	1.11	47	0.185	0.052	1.3
11	"	"	0.221	0.231	0.010	1.05	50	0.212	0.019	1.1
12	"	"	0.334	0.480	0.146	1.44	.....	.....	.....	.....
13	Healthy	$1\frac{1}{2}$ hrs. After 100 Gm. Grape Sugar	0.093	0.075	0.018	0.80	47	.....	.....	.....
14	Drinker	$1\frac{3}{4}$ hr. After 100 Gm. Grape Sugar	0.111 0.113	0.126	0.014	1.13	.....	.....	.....	.....
15	Arteriosclerosis	2 hrs. After 100 Gm. Grape Sugar	0.056	0.058	0.002	1.03	46	0.054	0.004	1.07
16	"	"	0.092	0.086	0.006	0.94	40	0.100	0.014	0.86
17	Carcinoma Liver	"	0.111	0.129	0.018	1.16	37	0.081	0.048	1.6
18	Liver Cirrhosis	"	0.142	0.188	0.043	1.30	36	0.051	0.142	3.8
19		"	0.145	0.193	0.051	1.36		0.070	0.118	2.7
20	Diabetes	"	0.180	0.225	0.045	1.25	37	0.103	0.122	2.2
21	Acromegalia	"	0.206	0.240	0.034	1.17	30	0.127	0.113	1.9
22	Diabetes	"	0.295	0.312	0.017	1.05	47	0.277	0.035	1.1
23	"	$2\frac{1}{4}$ hrs. After 50 Gm. White Bread	0.126	0.126	0	1.00	31	0.126	0	1.0
24	"	$2\frac{1}{4}$ hrs. After 100 Gm. White Bread	0.325	0.344	0.019	1.06	41.5	0.300	0.044	1.1
25	"	3 hrs. After 150 Gm. White Bread	0.428	0.386	0.042	0.90	45	0.480	0.094	0.8
		4 hrs. After 50 Gm. White Bread	0.234	0.228	0.006	1.00	42	0.243	0.015	0.9

made one hour after carbohydrates were ingested, the whole blood was higher in sugar than was the plasma. In cases 13 and 24 the differences were so great that they could not possibly be due to errors in technic or calculation. In one case the sugar concentration in whole blood went up and that of the corpuscles

diminished, due to the fact that the corpuscles must have yielded up some of their sugar. This phenomenon was first noted by Rona and Takahashi[17] and E. Frank and Bretschneider.[18] Tachau also claims that the increase in sugar concentration in the corpuscles, observed in alimentary hyperglycemia, was due to the relative permeability-increase in vitro in human corpuscles for grape sugar, as suggested by Rona and Doblin, Hoeber and Masing.[19] We can think of it in this way: when the alimentary hyperglycemia begins and sugar is thrown in increased quantity into the circulation, it is first dissolved in plasma and penetrates the corpuscles secondarily. As the hyperglycemia declines, the sugar content of the plasma goes down and the corpuscles then throw their sugar in excess into the plasma. Tachau attempts to explain by this line of reasoning why it is that in the presence of a declining hyperglycemia of alimentary origin, the serum loses its sugar, and strange to say, the corpuscles then hold more sugar than the plasma. Perhaps this is due to a sudden liberation of sugar from the blood stream. In this connection the work of E. Masing[19] bears strongly on this point: he showed by exhaustive experiments that the addition of sugar to a quantity of blood in vitro is followed by the taking up of the sugar by the corpuscles first; then later the corpuscles give up this sugar excess and in the final analysis sugar in larger quantities is found in the plasma. This is in confirmation of the work of Rona[20] and the prior publication of Masing.[21] Masing further showed that the addition of sugar to blood was followed by the

TABLE IV

INVESTIGATIONS ON THE SAME PERSONS AND EXPERIMENTAL ANIMALS

Case No.	Diagnosis	Remarks	SUGAR CONTENT					
			Blood Volume %	Whole Blood %	Plasma %	Corpuscles %	Difference Between Plasma and Corpuscles %	Quotient Plasma / Corpuscles
1	Nephritis.....	Fasting 1 hr.	45	0.111	0.126	0.093	0.033	1.3
		After 100 Gm. Grape Sugar..		0.142	0.155	0.125	0.033	
2	Diabetes.....	Fasting 1 hr.	50	0.129	0.138	0.120	0.018	1.2
		After 50 Gm. White Bread..		0.221	0.231	0.212	0.019	1.1
3	"	"	....	0.243	0.246	....	....	....
				0.334	0.480			
4	"	Fasting 2 hrs.	47	0.150	0.173	0.125	0.048	1.4
		After 100 Gm. Grape Sugar..		0.295	0.312	0.277	0.035	1.1
5	"	Fasting 1 hr.	41.5	0.361	0.387	0.325	0.062	1.2
		After 100 Gm. White Bread..		0.325	0.344	0.300	0.044	1.1
6	Dog 1, Police Dog.....	Fasting ¾ hr.	43	0.081	0.090	0.070	0.020	1.3
		After 100 Gm. Grape Sugar..		0.213	0.264	0.146	0.118	1.8
7	Dog 2, Bull Dog	Fasting 1 hr.	36	0.081	0.087	0.069	0.018	1.3
		After 80 Gm. Grape Sugar..		0.150	0.159	0.133	0.026	1.2
8	"	Fasting 1¼ hrs.	36	0.082	0.087	0.072	0.010	1.1
		After 120 Gm. Grape Sugar..		0.223	0.174	0.311	0.137	0.6

slow entrance of sugar into the corpuscles at zero Centigrade, faster at 25° C., and that this entrance was hindered markedly by high temperatures. Masing also showed that treatment of corpuscles with formalin enhanced their permeability for sugar.

The fourth table of Tachau's data on the same persons and on experimental animals, eight cases in all, showed a great difference between the sugar content of plasma and corpuscles in case 4, a diabetic, blood taken two hours after 100 grams of grape sugar were administered. The difference was 0.048 per cent. The greatest difference was in case 5, 0.062 per cent, a diabetic, fasting one hour after 100 grams of white bread were ingested. It is significant to note that the three dogs examined, cases 6, 7, and 8, showed about the same percentage of blood sugar in their whole blood: viz., 0.081, 0.081, and 0.082 per cent, respectively. The least difference found between plasma and corpuscles was in case 8, 0.010 per cent, and the greatest in case 5, 0.062 per cent.

Our figures are based upon a comparison of the blood sugar content of 24 cases, using the latest method, that of Benedict and Lewis[22] modified by Myers

TABLE V

ANALYSIS OF WHOLE BLOOD, PLASMA AND CELLS

NO.	NAME	SEX*	DATE	WHOLE BLOOD	PLASMA	CELLS	REMARKS
1	W.M....	♂	8/8	0.135	0.135	0.135	Patient normal. Blood taken after breakfast.
2	F.B.....	♂	8/15	0.132	0.129	0.132	Patient normal. Blood taken after breakfast.
3	Dr.H....	♂	8/16	0.204	0.204	0.200	Patient diabetic.
4	M.H....	♂	8/18	0.156	0.153	0.156	Patient epileptic.
5	Dr.H....	♂	8/19	0.165	0.162	0.165	Patient fasting six days.
6	C.B.....	♂	8/22	0.159	0.155	0.159	Patient syphilitic. Blood taken after dinner.
7	F.H.....	♂	8/22	0.140	0.138	0.140	Patient syphilitic. Blood taken before breakfast.
8	E.B.....	♂	8/23	0.300	0.225	0.240	Case of a boy of 12 years who was dying at the time blood was taken (DIABETIC COMA).
9	B.E.S...	♂	8/31	0.132	0.129	0.132	Blood taken after injection of salvarsan.
10	J.W.....	♀	9/13	0.200	0.196	0.196	Patient diabetic.
11	Dr. H...	♂	9/16	0.225	0.225	0.225	See case No. 3 and 5.
12	9697....	♂	9/18	0.135	0.135	0.132	Patient syphilitic. Wassermann++++
13	G.M....	♂	9/18	0.123	0.123	0.123	Patient syphilitic.
14	M.R....	♀	9/20	0.345	0.340	0.340	Patient diabetic.
15	E.G.....	♂	9/21	0.144	0.144	0.144	
16	J.R.....	♀	10/3	0.120	0.120	0.120	Patient on "ALLEN TREATMENT" since 9/27.
17	J.R.....	♀	10/19	0.129	0.129	0.129	See Case No. 16.
18	M.M....	♀	10/20	0.090	0.090	0.090	Patient pregnant and has only one kidney.
19	F.S.....	♂	11/10	0.102	0.099	0.102	Patient normal. Blood taken after breakfast.
20	M.S.....	♀	11/20	0.138	0.138	0.138	Patient on "ALLEN TREATMENT" since 11/17.
21	G.D.....	♀	11/21	0.102	0.102	0.099	Patient syphilitic and has a trace of sugar in urine.
22	P.R.....	♂	11/21	0.087	0.084	0.087	Blood taken one hour after injection of salvarsan.
23	M.G.....	♂	11/25	0.210	0.207	0.210	Patient syphilitic.
24	B.P.....	♂	11/25	0.120	0.120	0.117	Patient diabetic.

* ♂ Signifies Male.
♀ Signifies Female.

and Bailey.[23] The blood was diluted one to five with distilled water, immediately after withdrawal, precipitated with picric acid, mixed with a stirring rod, and allowed to stand with occasional stirring. The tube is now centrifuged for a few minutes and the supernatant fluid filtered into a dry test tube through a small thick piece of filter paper. Three c.c. of the filtrate are pipetted into a specially graduated test tube, 1 c.c. of 20 per cent sodium carbonate added, and the solution heated for fifteen minutes for the development of color. The solution is allowed to cool, made to volume with water, 10, 15, or 20 c.c., dependent upon depth of color, mixed and compared in the Hellige colorimeter with the wedge of standard picramic acid. In these determinations, of course, we worked with another part of the same blood, which was strongly centrifuged beforehand, in that way separately gathering the plasma and the cells. The plasma and the cells in turn were handled in the same way as was the whole blood.

Table V shows the results of our own investigations.

From a study of these twenty-four examinations it can be readily seen that the quantity of sugar in the whole blood, in the plasma, and in the corpuscles in nearly all cases is the same. Our cases were normal individuals, syphilitics, diabetics before and after undergoing "Allen" treatment, and one epileptic. Our figures agree rather closely with those of Tachau already cited at length. In but one case, No. 8, did we see a wide variation from this agreement: here we had 0.30 per cent in the whole blood, 0.225 per cent in the plasma and 0.24 per cent in the cells. This was a very interesting case of a boy of twelve years who died within twenty-four hours after admission into the City Hospital of what was judged to be diabetic coma. The thought suggested itself that in the terminal stages of life, in diabetes, there is perhaps a variation in the sugar content of the various parts of the blood, but as yet we have had no opportunity in diabetic coma cases to verify this observation.

CONCLUSION.

Using the latest methods of sugar analysis in blood, namely, that of Lewis and Benedict as modified by Myers and Bailey, we find that the amount of sugar is practically the same in the whole blood, plasma, and cells. This is in the main in perfect agreement with the work of Tachau who used the older technic of sugar estimation. This seems to disprove the theoretical views of some of the older physiologists who held that a part of the sugar in the blood was in a state of loose combination with some other substance. This obsolete idea has, of course, already been considerably shaken by the work of Rona and Michaelis[24] who showed that blood sugar is in a state of solution; they showed that when diluted blood is shaken with certain colloids, such as ferric chloride or kaolin, the proteins form a colloidal combination, and are absorbed. They can then be quantitatively precipitated by the addition of a trace of electrolyte, but no trace of sugar is removed from the solution by this treatment. If the sugar were united with the proteins it would be carried down with them, and as the reagents used cannot have any disruptive effect, it is not possible for the sugar to exist in combination with the proteins. As Cammidge[25] states, too, another piece of evidence in support of the free state of dextrose in the blood is

furnished by the observation that, whereas charcoal absorbs both sugar and protein when shaken with a solution containing these two substances, yet it absorbs the protein, but not the dextrose, when acetone is present. The acetone being more absorbable than the dextrose, prevents the latter being taken up by the charcoal. Further evidence is also furnished by the results of dialysis experiments.

BIBLIOGRAPHY.

[1]Tachau, H.: Ztschr. f. klin. Med., 1914, lxxix, 421.
[2]Lépine: Le diabète sucré, 1909.
[3]Michaelis and Rona: Biochem. Ztschr., 1909, xvi, 60.
[4]Hollinger: Biochem. Ztschr., 1909, xvii, 1.
[5]Rona and Döblin: Biochem. Ztschr., 1911, xxxi, 215.
[6]Frank, E.: Ztschr. f. physiol. Chem., 1911, lxx, 135.
[7]Lyttkens and Sandgreen: cited by Bang: Der Blutzucker, 1913.
[8]Hoeber: Biochem. Ztschr., 1912, xlv, 207.
[9]Schirokauer: Berl. klin. Wchnschr., 1912, No. 28.
[10]Masing, E.: Pflueger Arch., 1912, cxlix, 227.
[11]Loeb: Biochem. Ztschr., 1913, xlix, 413.
[12]Michaelis and Rona: Loc. cit.
[13]Frank, E.: Loc. cit.
[14]Rolly and Oppermann: Biochem. Ztschr., 1913, xxviii.
[15]Tachau: Deutsch. Arch. f. klin. Med., 1911, cii, 600.
[16]Lépine: Le diabète sucré, 1909.
[17]Rona and Takahashi: Biochem. Ztschr., 1911, xxx, 99.
[18]Frank, E., and Bretschneider: Ztschr. f. physiol. Chem., 1911, cxxi, 157.
[19]Masing, E.: Pflueger Arch. f. Physiol., 1914, clvi, clvii, No. 8, 401.
[20]Rona: Biochem. Ztschr., xxxi, 215.
[21]Masing, E.: Pflueger Arch. f. Physiol., cxlix, 227.
[22]Benedict and Lewis: Jour. Biol. Chem., 1915, xx, 61.
[23]Myers and Bailey: Jour. Biol. Chem., 1916, xxiv, No. 2, 147.
[24]Rona and Michaelis: Biochem. Ztschr., 1909, xiv.
[25]Cammidge, P. J.: Glycosuria and Allied Conditions, Longmans, Greene & Co., 1913, 19.

LABORATORY METHODS

A NEW KIND OF LONG PAPER KYMOGRAPH*

By D. E. Jackson, Ph.D., M.D., St. Louis, Mo.

ALMOST the entire framework of the long paper kymograph† shown in the accompanying illustration is made of regular standard gas pipe and fittings. Three sizes of pipe (and fittings) are used in the construction of the base or supporting table, viz., 1, 1¼ and 1½ inches. The main horizontal shaft to which the drums are attached is constructed of inch piping except in the middle portion where 1¼ inch sized fittings are used in order to make a stronger hinge. The small frames to hold the drums, etc., are of smaller piping (¾ or ½ inch).

When the "lock pin" is pulled out (upwards) the entire drum shaft and drums can be turned over (backwards in the illustration) downwards until the center of the horizontal shaft rests on the "smoking rest arm." The drums are then horizontal and in the correct position for smoking the paper which is pasted together at the ends and passed like a belt around the drums. The left hand drum is free to turn. The belt is dropped off one of the pulleys at the right hand drum and then the "crank for smoking" is used to turn the right hand drum rapidly to smoke the paper.

The paper passes along just below (outside) the smoking plate. This plate is made of cast iron and is 12 by 6 by ¼ inches in dimensions. The smoking flame is held just below the smoking plate which keeps the paper from getting too hot.

The left hand drum is held in a frame which slides on two parallel (1 inch) pipes which extend from the smoking plate to the left hand end of the horizontal shaft. The fittings marked "drum slide" and "B" are bored out on the inside to slide over the parallel pieces of piping.

The drums may be made of sheet brass rolled to fit the heads (ends) which can either be made of cast brass or simply cut (turned) from a sheet of brass (about ⅛ inch thick, or galvanized sheet iron 1/16 inch thick). It is much cheaper, and perhaps better in every way, to make the cylindrical portions of the drums of galvanized sheet iron (1/16 inch thick). These pieces can easily be trimmed to the proper size and rolled into perfect cylinders at a very nominal cost by any sheet metal company. The parts of the drums are easily soldered together.

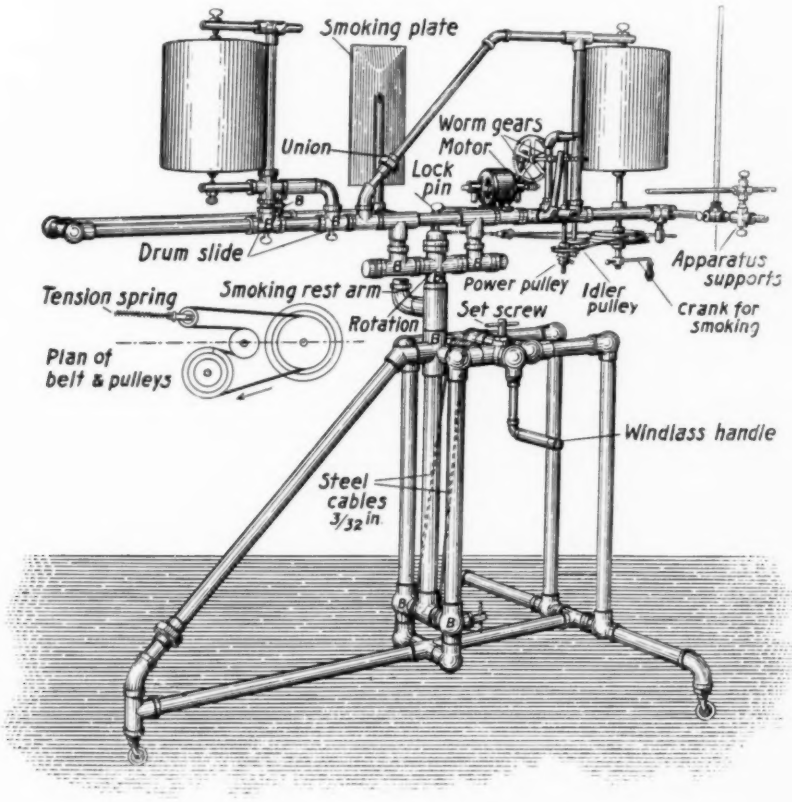
A small motor (costing $3.50 and purchased, second hand, of the Gregory Electric Company, 16th and Lincoln Streets, Chicago) has been used to turn the drums. The motor is 1/20 horse power and revolves 1500 times per minute (Robbins and Myers).

It may perhaps be advisable to use a slightly stronger motor (1/16 horse

*From the Department of Pharmacology of Washington University Medical School, St. Louis.

†The kymograph here described was demonstrated in New York on December 29, 1916, before the Federation of American Societies for Experimental Biology.

power) if some difficulty is experienced in getting gear wheels that are entirely satisfactory. I have used two sets of worm gears as indicated in the picture. The large gear wheel may have from 120 to 140 teeth (motor 1500 R.P.M.) and the smaller wheel (horizontal) has 84 teeth. But for motors of different speeds these gears must be bought to give the desired drum speeds. A large number of different speeds may be secured for the drum by having several different sized pulleys (which are interchangeable) to place on the lower end of the power spindle (power pulley) and on the lower end of the drum spindle. These spindles are all made of ½ inch mandrel iron (*shafting*). The pulleys



Motor driven long paper kymograph. (For description see text.)

are attached by set screws and can be readily removed or interchanged. The round leather belt is kept taut by a tension spring (see plan of belt and pulleys).

Adjustable and interchangeable apparatus supports are attached to the right hand end of the horizontal shaft.

The main horizontal shaft and the drums are supported by a sliding upright shaft of 1½ inch piping. At the lower end of this shaft is placed a tee which connects with two more side-outlet tees which are bored out (marked B) to slide on the two outer upright bars. A set screw is shown in one of these side-outlet tees. The set screw is used to fasten the sliding tee at any desired height.

The central supporting shaft also slides through another side-outlet tee at the upper part of the table. The portions of the hinge marked "B" are also bored out to rotate on the short pieces of piping which pass through the fittings.

The drums are raised or lowered by means of a windlass and a steel cable (3 32 inch in diameter). The cable is fastened to the lower end of the supporting shaft and passes upward (double) to the windlass which can be locked by a set screw. The fittings through which the roller of the windlass passes are bored out to permit rotation of the roller (1¼ inch piping) as the crank is turned.

The main horizontal drum shaft and drums can be rotated in a horizontal plane around the central axis. This is accomplished by the use of a cross, the perpendicular openings of which are bored out to turn on the upper end of the main supporting shaft (at the point marked "rotation" in the illustration). On the extreme upper end of the main supporting shaft is placed a cap. Through this cap the lock pin passes down from the two holes in the horizontal shaft above. If the lock pin is pulled out and the cap screwed off, then the entire upper part of the kymograph can be lifted off from the base. This is a matter of great convenience for shipping, etc.

Castors (as shown in the picture) are not necessary and are perhaps best avoided for ordinary routine purposes.

The two drums here shown are 10 inches high and 8 inches in diameter. For those who so prefer it is a very easy matter to attach a small extra drum (or cylinder) to the frame which holds the right hand drum. This extra drum can be used to furnish a *flat* recording surface for the writing points of the tambours, etc. This is accomplished by passing the belt of drum paper around all three drums.

"Ball" pattern fittings (railing fittings) have been used in a number of places in the construction of the framework because they are somewhat neater in appearance than the ordinary styles of fittings.

The kymograph here shown and described should be considered merely as typical of the many forms and sizes of kymographs which can easily and cheaply be constructed of the materials here indicated. For the particular kymograph illustrated here the cost of materials, including the labor of a steam and gas fitting company, which made up the base and the main portions of the upper part of the framework, and the labor of another firm which rolled and soldered the brass cylinders, was a little more than $40.00. But this price is higher than it should probably have been because a little "experimenting" had to be done in the work of construction. The attachment of the motor, gears and pulleys, etc., represented a further small expense.

In many schools it should easily be possible to have a sufficient supply of these long paper kymographs constructed either in the departmental shops or in other departments in the school in which mechanical work is being taught. In these cases, if a plumbing company can be hired to make the framework, and a sheet metal company to roll the drums, the total cost of construction should not exceed $75.00 and might be considerably less. The belt pulleys should be made of cheap cast iron. The set screws can be obtained from the stock of any large hardware store. The gear wheels and worms cost about

$3.30. The motor must be bought to fit the current available in the laboratory, and the switch used for starting or stopping the motor can be placed on an extension cord, thus allowing the operator to control the drum from any place about the room. A spinning device for the right hand drum can easily be attached to the drum spindle.

THE CONSTRUCTION OF CLINICAL LABORATORY EQUIPMENT

By Miles J. Breuer, M.D., Lincoln, Neb.

THERE are many practitioners who would be desirous of availing themselves of the modern clinical laboratory methods, were they not deterred therefrom by the high cost of equipping an efficient laboratory. If a practitioner is to have his own laboratory, he must have the various time- and labor-saving devices; otherwise, the routine performance of many laboratory procedures would be out of the question for a man who has patients to take care of. Yet, the cost of this apparatus is so high as to make it out of the reach of many. The writer wishes to show how some of these pieces of apparatus may be constructed by the physician himself.

These remarks will appeal especially to the young man in practice, who has been trained in the modern scientific methods, and is eager to apply them for the benefit of his patients, yet finds in the course of the doctor's rather difficult beginning-period, that he cannot afford to purchase the needed apparatus. He is the very one who will have the time that it takes to construct the apparatus.

In fitting up the laboratory, a good microscope is the biggest item of expense, and of course, the purchase of this is unavoidable. The various small pieces of glassware are not a great item of cost, with the exception of the hemocytometer. The first thing on which the physician can increase his efficiency without great expense, is the centrifuge. A hand centrifuge can be purchased at a moderate price, but this is useless for anything except urinalysis, and very slow and laborious for this purpose. With an electric centrifuge, one can be attending to other tasks while the specimen is whirling, and cut down the time spent in the laboratory, by a great deal. A good electric centrifuge is rather expensive. The writer's first electric centrifuge was a discarded electric-fan motor, on which were fastened the arms and shields from a hand centrifuge, and this performed excellent service for a long time. Small electric motors to run on the 110-volt current can always be procured at second-hand; in most large cities there are regular exchanges handling them, and a very few dollars paid for one of these, will be many times repaid by the added convenience and efficiency for the man who feels that the price of an electric centrifuge is too much for him.

The next piece of apparatus of importance is an incubator. The lack of an incubator is the only thing that stands in the way of many men doing for them-

selves certain very useful things, such as diphtheria cultures and autogenous vaccines. The making of an electric incubator and thermostat is very simple; and once adjusted, the apparatus requires no care, except to switch it on and off when required, like an electric light.

Our incubator was made by starting with a cubical frame, a foot on each edge, constructed of 1-inch sticks; ¼-inch cypress boards were used to cover this, both inside and out, thus making a double-walled box with a 1-inch space

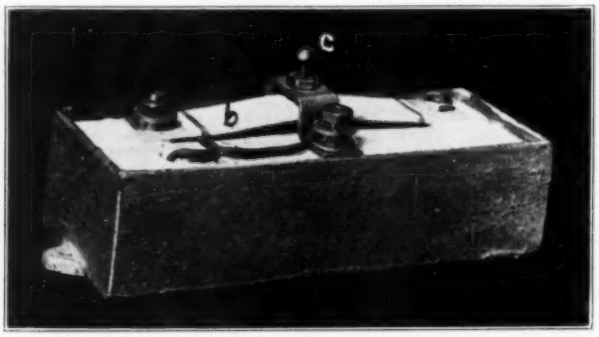


Fig. 1.—Window flasher, original form.

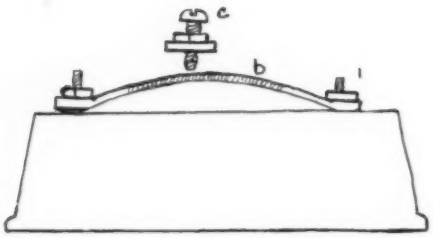


Fig. 2*A*.—Original form of "flasher."

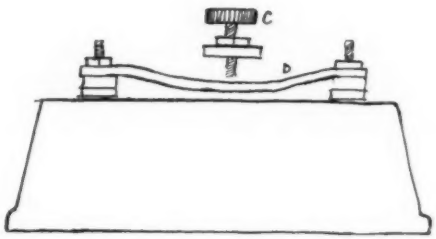


Fig. 2*B*.—"Flasher" altered for thermostat.

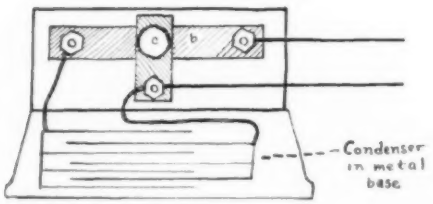


Fig. 2*C*.—Connections of thermostat.

between the walls. In this space were wrapped several thicknesses of newspaper (newspaper being one of the best heat-insulators we have). An inner glass door was provided and an outer wooden one. The details of construction are not given, as they are trivial, and may be left to anyone who makes such a box. The heat is furnished by a carbon-filament incandescent lamp. The carbon filament produces much more heat than the tungsten. The heating is rendered much more even if an ether can with the top and bottom melted off, mounted on three wire legs, is placed around the incandescent globe. The globe

may be dipped in shellac colored red with carmine, eosin, or safranin (fuchsin will not do, as it changes color) to protect the cultures from the bactericidal effect of white light. A snap switch is mounted on the outside for turning the heat on and off. Where the electric wires pass through the wall of the incubator, the holes should be protected by porcelain bushing. A thermometer is suspended through a hole in the top, and rendered tight by putting it through a cork stopper.

The thermostat was made from one of the "window flashers" which is shown in Fig. 1, and which can be purchased at any electrical supply store. Such a piece of apparatus would not be difficult to make, but this was chosen for

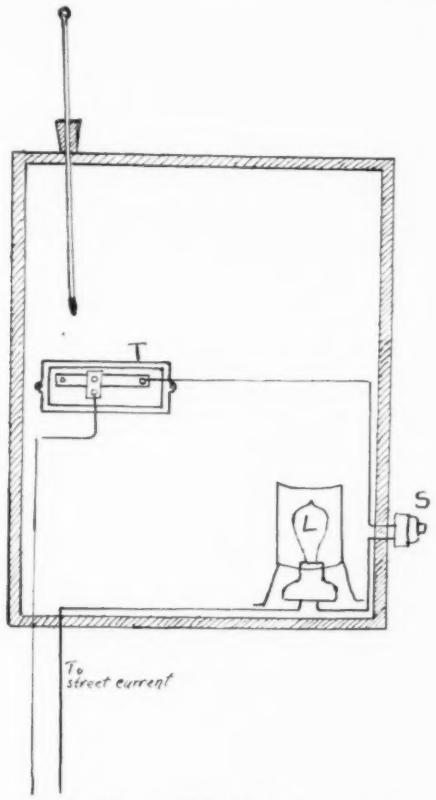


Fig. 3.—Diagram of incubator connections.

the reason that it met with the underwriters' approval, which a "homemade" piece of apparatus would not, even though it might be even more safe and efficient. There is a great difference in price between this apparatus and a regular electric thermostat for incubators.

The curved metal bar *b*, on the porcelain base, is wrapped with fine insulated wire. This is not required for our purpose, and should be disconnected and cut off. The bar is then reversed, so that the convexity is downwards instead of upwards. In order to effect this, it is necessary to block up the ends by means of two screw-nuts. Bolts and nuts should fit very tight; if they do not, larger ones should be put in, as everything depends on the ends of the bar being

fixed absolutely rigid. The expansion of the bar increases the degree of convexity, depresses the middle portion and breaks the contact when the proper degree of temperature has been reached. The platinum contact may be transferred from one side of the bar to the other, by a jeweler if one cannot do the work oneself, or the bar may be simply bent in the other direction. For a long time we used our apparatus with the original contact-screw *c*, but later replaced it with one having a large milled head and a finer thread, making adjustments finer and more accurate. The wires that extend into the porcelain block connect

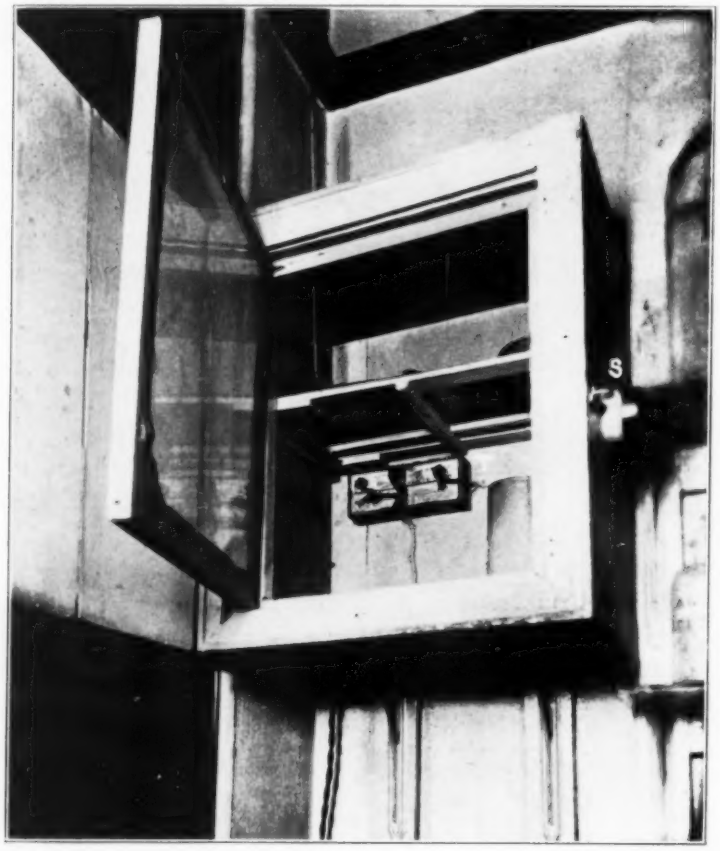


Fig. 4.—Incubator.

with the condenser, which prevents excessive sparking, and which should be connected as shown in *C*, Fig. 2. The connections for the incubator are shown in Fig. 3.

To adjust the thermostat, the contact-screw *c* is set down far enough to maintain a contact. The thermometer is watched through the glass door, keeping the outside door closed between observations. When the temperature has reached 37.5 degrees C, the door should be opened and the contact-screw quickly turned to the left until the light goes out and no further. The setting of the screw may have to be repeated to make the adjustment accurate; the idea is to have

the circuit broken at the exact instant that the temperature reaches 37.5 degrees C. If the box has been tightly made, if the bar on the thermostat is rigidly mounted, and the set-screw *c* is steady, this incubator will hold its temperature accurately and without attention.

The next two pieces of apparatus described are not strictly necessary in the practitioner's laboratory, but they effect such a saving of time that the trouble taken in their construction is many times repaid. In the performance of the complement-fixation test, it is the inactivation and the incubation which the man in practice finds most irksome; watching a water-bath with a thermometer in one hand an a Bunsen-burner in the other, consumes so much time that he is

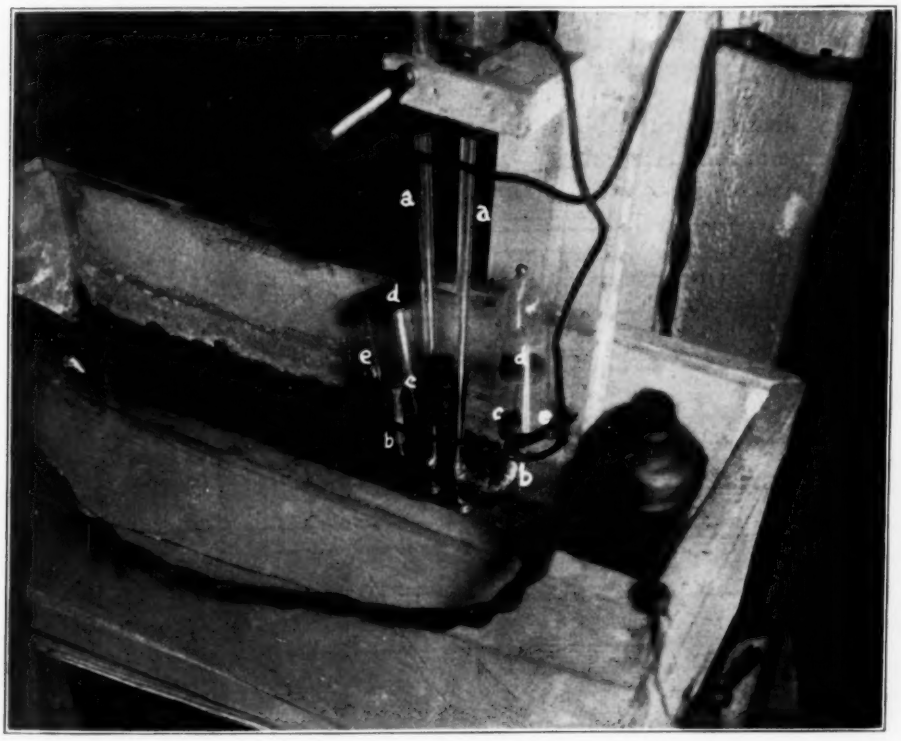


Fig. 5.—Water-bath.

unwilling to do the work. By means of an automatic water-bath, these portions of the process may be left to take care of themselves; and if there is a good centrifuge, there is no excuse for anyone's not performing his own complement-fixation tests. The same applies to the shaking-machine. Our shaking-machine, though originally made to aid in shaking up autogenous vaccines, now finds numerous other applications, as in shaking blood-counting pipettes, sputum for concentration methods of search for tuberculosis bacilli, urine-Obermayer mixtures for the indican test; in fact, every day we find a new use for it. To a busy man, the shaking-machine and the automatic water-bath may mean the whole difference between being able to make his own autogenous vaccines, and not being able to do so; with these two pieces of apparatus, all that will be required of

him is the transplantation of the cultures, and timing when in the various apparatus.

Our water-bath was made from a galvanized-iron box, 6x6x14 inches (constructed for us by a tinner), with a pet-cock in one end by means of which the water can be drained out when desired. A wooden box was built around this, with newspapers between. The heat to the water is supplied by two carbon-filament incandescent globes. Some of the immersion water heaters on the market were tried, but were found to remain hot too long after the current was turned off, causing the temperature to mount too high. (We are contemplating the construction of a special heating unit for this purpose.) The electric globes are sufficient for this purpose, if the glass part is kept submerged in the water by means of wire racks.

The thermostat is the most difficult portion of the apparatus to make. If one is not versed in glass working, the glass portion may be procured to order from any house which handles glass apparatus; and the cost ought to be small, as the apparatus is very simple. However, as it involves only the simplest elements of glass working, there are many who will be able to make the apparatus themselves. The writer is of the opinion that anyone who attempts to work in a laboratory ought to be acquainted with at least the rudiments of glass working and blowing. There is nothing difficult about it. A small gasoline torch will supply sufficient heat; or blasts may be purchased for a small sum. Fig. 9 shows a blast which the writer constructed himself from an old Bunsen burner, by soldering a small brass tube inside, discarding the base, soldering up the side openings tight, and hammering the end (B) until shaped as shown. Gas is connected at G, and compressed air (with which our building is equipped) at A. (The pump-tanks used in nose and throat work for atomization will furnish all the pressure necessary). It will certainly be worth any laboratory worker's time to spend his spare moments for a couple of weeks experimenting with glass tubing, in the ability gained to fashion various minor pieces of apparatus for himself.

The thermostat (A and B, Fig. 6) consists of a small bore tube, of about 1 mm. internal diameter and 20 cm. long; the actual diameter being immaterial, if it is small enough to afford considerable rise and fall of the mercury on change of temperature. A bulb is blown on the end of it, keeping in mind that it must be constantly rotated, in the flame and out of it; and one must blow only a little at a time, and do so repeatedly, in order that the walls of the bulb may become thick enough to stand the weight of the mercury. One or two cm. above the bulb, a small opening is blown in the side of the tube, by making the flame very small and pointed, and carefully blowing out the softened glass. Another tube (which must be prepared beforehand) has the edge on one end thinned by blowing a bulb so rapidly that it bursts, and breaking or filing off any flanges that project more widely than the outer diameter of the tube. This thinned end is heated to redness at the same time that the long tube with the side-hole is kept in the flame; the two pressed together; and the thick portion where the joint is, blown gently outward to make the welding

thorough and decrease the thickness. The side arm can then be bent upward at a right angle. Bending is best done in the luminous gas flame.

The upper contact is made by inserting into the long upper capillary portion of the instrument, a wire tipped with platinum (a jeweler can solder the platinum). The side arm needs provision for adjusting the height of the mercury. A large nut *c* is hollowed out with a drill on one side so that the tube *b* fits into it. It is cemented to the tube with litharge and glycerin mixed so as to form a

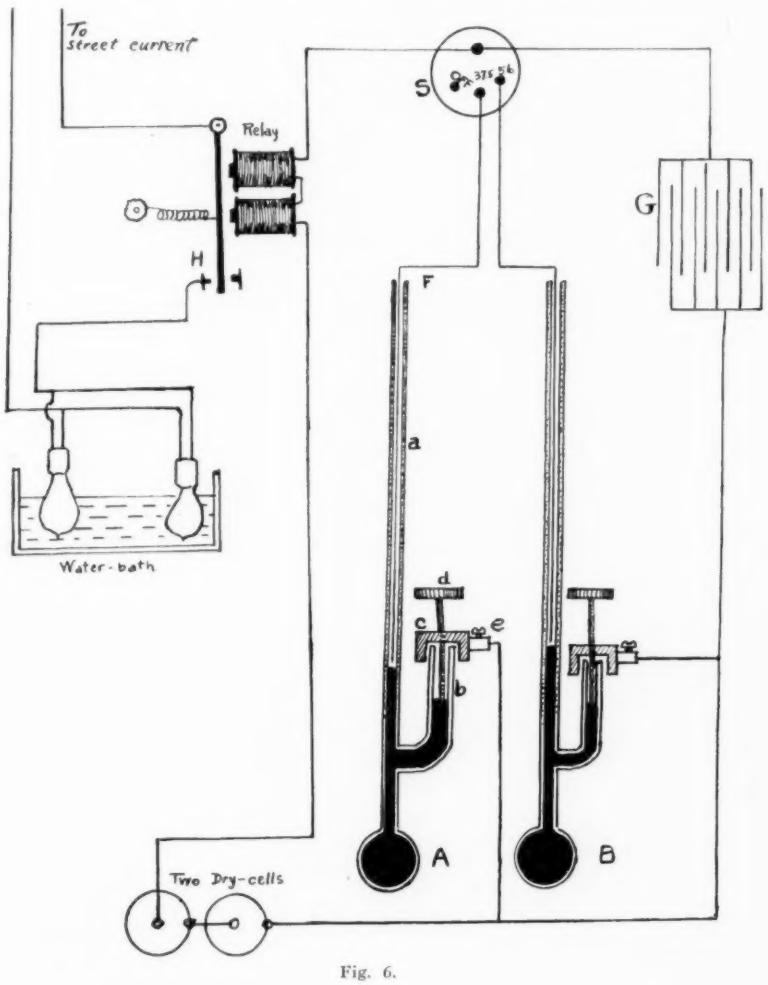


Fig. 6.

thick paste, and allowed to harden in a warm place for several days. A thumbscrew *d* to fit the thread of *c* and to enter the bore of the side arm *b* dips into the mercury and changes its height. A connector *e* for wire, is soldered to the nut.

Two such thermostats were made, in order that the two temperatures commonly used, 37.5 and 56 degrees centigrade could be available without readjusting the instrument each time; and by means of a three-point switch at S, the desired one can readily be thrown into connection. To prevent excessive spark-

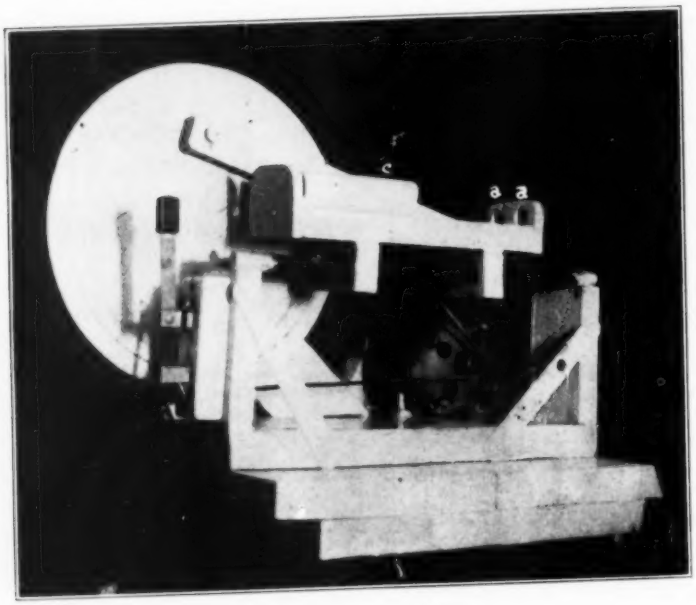
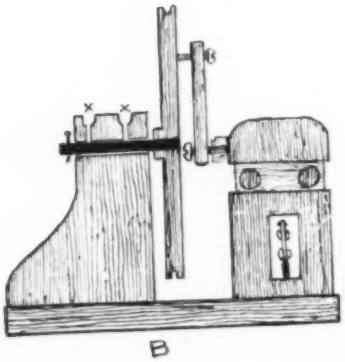
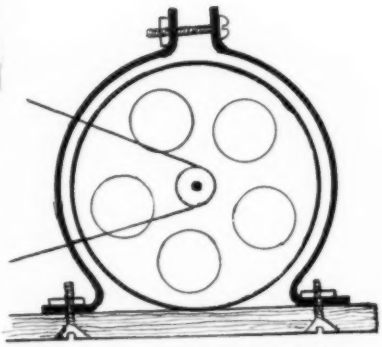


Fig. 7.—Shaking machine.

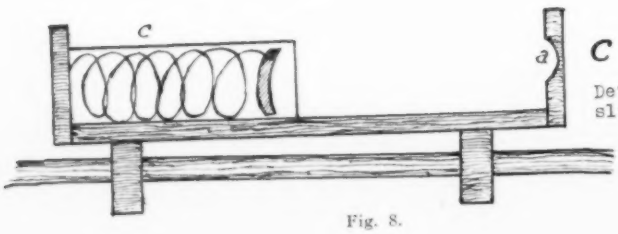


B

End projection of shaking-machine



A Method of mounting motor.



C

Details of sliding-bed.

Fig. 8.

ing on the mercury surface, due to the coils of the relay, a condensor *G* is thrown between the circuit-breaking terminals of the thermostat, as shown in the illustration. This was made from fifty sheets of tinfoil 3 inches square, interleaved with paraffined paper 5 inches square; the 1st, 3rd, and odd numbered sheets projecting over one end to connect together to one terminal, and the 2nd, 4th, and even numbered sheets over the other end for the other terminal. Care must be taken that there is no short circuit between the terminal (a current should not pass through the condensor).

A 20-ohm telegraphic relay operates the heating circuit. On the relay it will be necessary to reverse the position of the two contact screws which stop the motion of the armature on each side; the one with the hard-rubber tip goes on the same side with the coils, so that in a position of rest, the relay circuit is closed at *H*, and opens when a current is sent through the magnets. The wires from the thermostat circuit should be connected to the posts where the words "20-ohms" are found; the other two are for the heating circuit.

To adjust the thermostat, with the switch at *S* on the proper point, screw up the thumbscrew *d*, so as to break the contact of the mercury with the wire in the tube *a*, thus opening the heating circuit. Allow the temperature to reach the

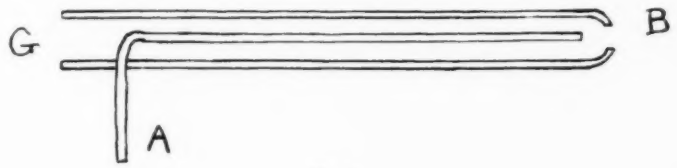


Fig. 9.

desired point (as 37.5 degrees C); then quickly screw down the thumbscrew, making the contact in the tube *a* between mercury and platinum-tipped wire. This closes the current through the relay, causing the latter to break the heating circuit, which remains open until the temperature of the water drops low enough so that the fall of the mercury breaks the thermostat circuit. This may be repeated for finer adjustment. It is advisable to have a cover fitted over the water-bath with openings for the thermostat tubes, as the bath keeps its temperature better and is more economical of current.

The construction of the shaking machine will be clear enough from the illustration, and will require but little explanation. The motor is from a barber's electrical hand-fan, which was obtained at second-hand. It is mounted on the baseboard by means of two iron straps, bolted to the baseboard and extending around the body of the motor, drawn together at the top by means of a stove bolt. The large pulley is of wood, 9 inches in diameter, with a rigidly fastened metal axle. This was procured from a planing mill. The small one is of brass and was procured at a machine shop. The belt is a leather string from a fly-net such as are used on harness in summer. The bearing of the axle of the large pulley is of oak and is three inches long in order to render the pulley steady. From above are bored two holes, widened at the top, to provide for lubrication, as in *B*, *x*, Fig. 8. The same provision is made on the crank-shaft, which is

pivoted to the large pulley and the sliding-bed. The crank-shaft should be as long as possible, to obviate the up-and-down shove on the sliding-bed.

The rods on which the sliding-bed is mounted, running through holes bored in cross-cleats on the under surface of the sliding-bed, are of ⅜ inch doweling, and are kept lubricated with graphite. The arrangement for holding the tubes which are to be shaken consists of two tin shields (*c*, Fig. 8) in the form of tubes, each containing a large coiled brass spring, faced with a concave block, *b*. The springs must be soft, and the coils wide, so that they can be compressed into a small space. Opposite each shield on the opposite end of the sliding-bed, is a depression, *a*, to hold the tube steady. Sizes and exact measurements are

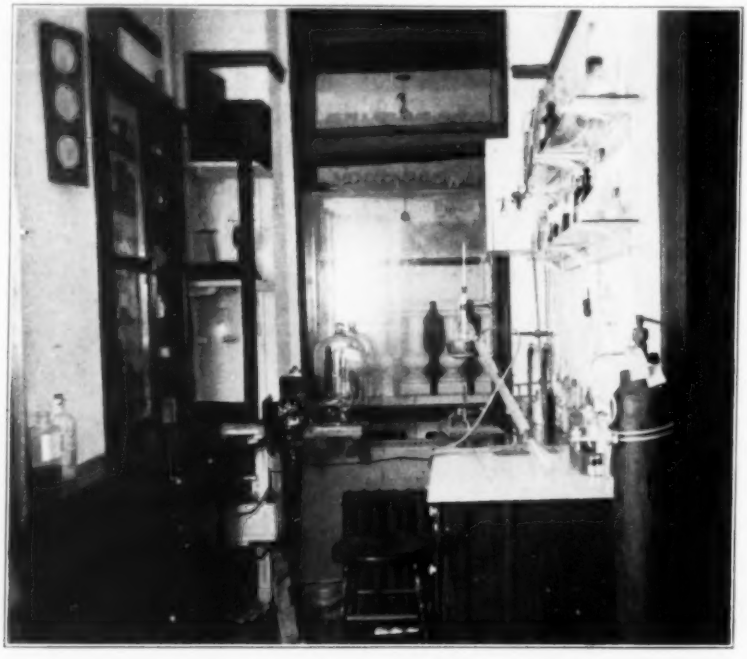


Fig. 10.—The laboratory.

not given, as each one will want to construct the apparatus of such a size as best adapted to his own needs.

Everything on this shaking machine with the exception of the motor and the two pulleys, was made by the writer during spare moments, and at very small cost. All of the pieces of apparatus described can be made at odd moments, which are as often as not wasted in some profitless form of so-called recreation. The work is of a character that will provide as much pleasure and recreation for the busy doctor—as much mental rest—as billiards or motion-pictures, provided only that he is interested. Once the apparatus is in his possession, he will find that he has within his reach practically all the laboratory methods performed nowadays as routine clinical procedures, and that the tests which he knows to be so invaluable in his work and which he once considered irksome drudgery, will now take but little of his time and will give him pleasure in their performance.

RAPID EXAMINATION OF OCCULT BLOOD BY THE BENZIDIN METHOD*

By Warren T. Vaughan, M.D.

STOOL examination, a laboratory procedure too often neglected by the general practitioner, and for very obvious reasons, is occasionally the source of a good clue in the diagnosis of morbid processes. It is only in well regulated hospitals, where various facilities make routine stool examination less onerous a task, and where there is always a young interne whose duty it is, and who has no choice in the matter, to examine them, that the procedure is consistently carried out on every patient. Quite obviously, every gastrointestinal case should be attacked from this angle, every ulcer or carcinoma suspect should be repeatedly examined. Occasionally a positive stool test for occult blood in a case, clinically not gastrointestinal, will suggest more thorough examination, and thus permit a correct diagnosis. Such, for instance, are cases of intestinal parasites and carcinoma, in which an early diagnosis is always so important. Barker[1] states that as much as 5% of blood may be present in feces without its presence being recognizable to the naked eye. A very satisfactory hospital routine to be followed, and one that should be strongly recommended to the general practitioner, is the routine examination of at least one stool from every patient, for occult blood, in addition to a simple microscopic examination of an unstained specimen. Of course, more frequent and more complete examinations should be made of stools from cases suspected of having gastrointestinal lesions, whether blood is present or not.

The test most generally employed is the guaiac test, usually Weber's method. The benzidin test as usually described, corresponds in technic, but is considered more delicate; by some even *too* delicate. Perhaps the simplest guaiac test consists in rubbing up fecal material the size of a pea in 5 c.c. of glacial acetic acid; shaking the solution with approximately 10 c.c. of ether; pouring off the supernatant ether, after it has separated out; and overlaying the ethereal extract with freshly prepared tincture of guaiac, to which about one-half its volume of hydrogen peroxide has been added. The blue or violet fading ring at the zone of contact is considered positive. The benzidin test, as generally performed, is done in essentially the same manner, or perhaps a little easier by the method described by Barker,[1] "A small piece of the feces is rubbed up with water; 3 c.c. of this unfiltered suspension is mixed with 2 c.c. of an alcoholic benzidin solution (concentrated by heat and filtered after cooling), and 2 c.c. of a 3% solution of hydrogen peroxide; a few drops of acetic acid are then added. If blood be present, an intense green color appears."

Grunwald[2] reports the relative delicacy of the various tests for occult blood as follows:

Weber's test (his own figures) -	positive with one part blood in	2000 dilution
Benzidin test - - - - -	positive with one part blood in	150000 dilution
Hemochromogla (spectroscopic test)	positive with one part blood in	5000 dilution
Potassium cyanide " "	positive with one part blood in	5000 dilution

*From the Medical Clinic of the Peter Bent Brigham Hospital, Boston, Mass.

Dewis,[3] in 1907, published an excellent review of the literature and a description of the comparative values of the different tests. He says that whereas many substances reacted positively to the original guaiac test, substances such as the oxide and subcarbonate of iron; ferrous salts, and iron sulphide; acetates, citrates and chlorides; the subacetate and sulphate of copper; and pus, all of which give a blue color of varying intensity, yet, as modified by Weber by the use of an acetic acid ethereal extract, most of the interfering substances, particularly the iron preparations, pus, saliva, milk, and starch no longer give positive reaction. Brandenburg[4] has shown that the positive reaction to pus is due to nucleoalbumin, and that it is precipitated by acetic acid. It is this precipitation by the acetic acid-ether extract that prevents the positive reaction to pus. It is found also that the acid-ether combination evidently prevents positive reactions for all common drugs. Weber found that of 67 medicines administered by himself to patients, none reacted positively with his guaiac test.

O. and R. Adler[5], in 1904, first described the benzidin color test for occult blood, and it is the method as essentially described by them that is positive with one part of blood in a dilution of one to one hundred and fifty thousand parts. This is indeed far too delicate for clinical work, and was so modified by Schlesinger and Holst[6] as to be much less delicate, and yet more sensitive than any of the other blood tests. According to their method, a saturated solution of benzidin is prepared by adding a knife-point of benzidin to 2 c.c. of glacial acetic acid in a clean test tube. This solution is preferably made fresh. A small piece of fecal material is stirred in a small test tube containing about 2 c.c. of water, after which it is brought to the boiling point to destroy the activity of enzymes. The benzidin solution is diluted about three times with commercial 3% hydrogen peroxide. Two or three drops of the boiled feces suspension are transferred to about 3 c.c. of the benzidin-acetic acid-peroxide mixture. The presence of blood is soon indicated by the development of a beautiful green, blue-green, or blue color.

Dewis, in using the test, remarks concerning the question of enzymes giving a positive reaction, that "in 22 specimens of stools, 10 benzidin tests were negative, and the remainder positive. No definite green color change was found *before* boiling, that was absent *after* boiling the specimen; but in nearly all the 10 negative tests, color change was wholly absent without boiling, proving that enzymes seldom, if ever, interfere with the benzidin test in stools." He feels that in testing gastric contents, it would be safer to always boil first as directed in the test.

Wagner[7], in 1914, recommended the so-called "dry test" for blood—a test which is reliable and extremely simple. He uses as reagent a knife-point of benzidin (powdered)—an amount the size of a match head—2 c.c. of glacial acetic acid, and 20 drops of a 3% solution of hydrogen peroxide. A few drops of this mixture, poured over a little of the solid feces, on a clean glass slide, will produce a greenish-blue fading color if positive. We have found it most convenient to pick up a particle of fecal material the size of a match head on the end of a toothpick and spread it out somewhat on a microscopic slide. Viewed against a white background, the green color stands out strikingly well.

It appears within five or ten seconds, and persists a minute or more. The hydrogen peroxide need be only roughly two-thirds the amount of glacial acetic acid, and the latter only approximately 1 c.c. The only apparatus required is a short test tube for mixing the benzidin, acetic acid and hydrogen peroxide, and a medicine dropper. One or two drops of reagent mixture suffices. It should be freshly prepared, but the solution, or rather suspension, remains good for two or three hours. In lieu of a glass slide any white glazed paper will suffice,—a calling card does very well. If paper is used there is nothing to clean up afterwards.

As regards accuracy and delicacy of reaction, when several months ago we first started to employ this test, it was with the assumption that, the benzidin test being more delicate than the guaiac, a negative benzidin would certainly indicate absence of occult blood, whereas, a positive test should be checked up by a guaiac test. This procedure was carried out for some time; and, even when doing a guaiac test on all stools positive by benzidin tests, much less time was consumed than was formerly done in doing guaiac tests only. Almost invariably, positive benzidins were accompanied by positive guaiacs. In the few cases in which negative guaiacs were obtained, by careful "playing" with the guaiac test (varying the proportions of the components) a definite and strongly positive guaiac was always obtained. We found that varying the amount of guaiac, or of hydrogen peroxide frequently made a negative guaiac become positive to correspond with a positive benzidin. With attention to these points, then, the guaiac and benzidin tests almost always agree, and it was concluded that inasmuch as the guaiac test has more variables which appear to influence its reactivity, any discrepancy between the two tests points to the guaiac test as the incorrect one until it can be proved by repetition to be otherwise. A negative benzidin test is more reliable evidence of the absence of blood from the stools than is a negative guaiac test.

The question is frequently raised as to whether meat ingested, or muscle fibers in the stool will give a positive test for occult blood. Dewis and Cowie[8] both report that an ordinary meat diet rarely gives a positive Weber test. Cowie, however, obtained a definitely positive guaiac reaction after the ingestion of 10 gm. of scraped raw meat, but none from lesser amounts of meat. Lewis conducted a similar experiment in which he ingested a "hearty evening meal of very rare roast beef for two days, other meals being meat free." On the four succeeding days he limited his diet to eggs, salt codfish, potatoes, oatmeal, fish cakes, fresh mackerel, baked beans, bread, butter, tea and coffee. Twenty-four hours after the second meat ingestion, both Schlesinger and Holst's benzidin test and Weber's guaiac test were strongly positive. The benzidin test remained positive after the fecal suspension had been boiled. After forty-eight hours, the benzidin test (both boiled and unboiled) remained positive, while the Weber test had become negative. On the third day the benzidin test was positive (unboiled) and negative (boiled), while the guaiac remained negative. On the fourth day after rare beef ingestion, both benzidin and guaiac tests were negative. This series, besides demonstrating that if sufficient rare meat be ingested a positive test for occult blood will result, also shows very well the relative sensitiveness of the two tests employed.

In contrast to this is Medical case No. 5811, Peter Bent Brigham Hospital, a man admitted with a diagnosis of pernicious anemia, but who on thorough examination showed many additional symptoms, chiefly gastrointestinal, which complicated greatly the diagnosis. Of thirteen stools examined, all gave a negative benzidin test (Wagner), but showed large amounts of fat and many undigested muscle fibers on microscopic examination. The meat eaten, however, was not very rare. A characteristic stool was as follows:

December 28, 1916.

STOOL: Gray, unformed, pasty, shiny. No macroscopic blood, mucus nor parasites. *Microscopic:* Numerous sheaths of fatty acid crystals and, after heating with acetic acid, numerous fat droplets and needle crystals. Numerous muscle fibers with striations well preserved. No starch granules. No pus or ova. Bile + ($HgCl_2$ test). *Benzidin:* Negative (Dr. Koefod).

Medical case No. 5377, a case typically pernicious anemia, and with intermittent attacks of diarrhea shows very well the fact that Wagner's benzidin test does not react positively to either muscle fiber or pus:

September 30.

STOOL: Thin, brown, semisol'd. No macroscopic blood, pus or parasites. A *microscopic* search revealed no parasitic ova. No starch nor fat globules. No muscle fibers. *Guaiac* test, negative.

October 3.

STOOL: Brown, watery, no macroscopic blood or pus. No segments of worms. *Microscopic* examination reveals no ova or segments—no meat fibers. No blood. Much pus. *Guaiac* test, negative.

October 6.

STOOLS: The last eight stools showed negative *benzidin* test, and on *microscopic* examination, no pus or parasitic ova, but many meat fibers.

October 8.

STOOLS: Of four stools examined, two are unformed and two fluid. Two show some macroscopic mucus. *Color* golden yellow brown. *Microscopic* examination shows very many leucocytes in two of the four stools. *Benzidin* test, negative on all four stools.

October 10.

STOOLS: (Oct. 8, 9, 10) Pus in one of four stools. This is the only stool that has shown a *positive benzidin* test.

October 11.

STOOL: Brown, semisolid, no macroscopic blood, pus, or segments. No *microscopic* blood or ova. Very few meat fibers. Considerable pus. Some phosphates. *Guaiac* negative.

October 17.

STOOL: Semisolid, normal brown. No macroscopic blood, pus or parasites. *Microscopic* examination, few red cells, otherwise negative. *Benzidin,* plus.

November 6.

STOOL: Fluid brown, much thick mucus. One small blood streaked spot. No other gross abnormality. *Microscopically,* blood cells and epithelial cells—nothing else abnormal. Careful search for ova and segments revealed none. *Benzidin,* strongly positive.

November 8.

STOOL: Fluid, dark brown, foul-smelling. Much thin mucus. No macroscopic blood, pus, or parasites. *Benzidin,* negative.

November 17.

STOOL: Fluid, chocolate colored, no gross abnormality. *Microscopically,* nothing abnormal seen. *Benzidin* and *guaiac,* positive.

Dewis found that with the Schlesinger-Holst benzidin test the following substances when tested directly reacted positively: raw green vegetables (slight); raw wheaten flour, corn, oatmeal, potatoes, carrot, parsnips, and squash. When cooked, all of these were negative. All meats, cooked and uncooked, gave positive benzidin test. Raw fish, fresh, dry and pickled, and shell fish gave a positive reaction, which became much weaker after cooking. After ingestion of fish, the stool was usually negative, although sometimes positive after fresh fish. Eggs were negative. Blaud's pills were quickly positive, but after ingestion of thirty grains daily there was no positive reaction in the stools.

With Wagner's test, we have found that thoroughly cooked meat still reacts positively, whereas cooked vegetables (potatoes, carrots, tomatoes) are negative. Blaud's pills, tincture of ferric chloride, bismuth subnitrate, potassium iodide, extract of cascara sagrada, compound cathartic pills, and A B & S pills, all reacted negatively, when treated directly with the reagent mixture. The pills were all crushed previous to testing.

As the test is applied to urine examination, urine containing red blood cells, whether it be bloody or merely smoky, reacts positively, whereas, if there be pus alone, no green color is produced.

Since Wagner's dry method was instituted, Doctors Golden, Barrows, Koefod, and myself have used it on over eight hundred cases, with entire satisfaction. Probably the benzidin test *is* more delicate than the guaiac test, and it *may* react when a guaiac test definitely will not; but it is well demonstrated that, for clinical purposes, its reliability and greater ease of execution make it preferable to the guaiac tests, and a negative benzidin test is more reliable evidence of the absence of blood from the stool than is a negative guaiac test.

Gregersen[9], of Copenhagen, reported in 1916 an exhaustive series of tests for occult blood in the stools. He finds the test positive in practically all of the stools of cancer patients, positive in some stools of ulcer cases; but never constantly positive in the same patient; and constantly negative in uncomplicated chronic gastritis, achylia, colitis, simple dyspepsia and constipation, gall stones, cardiac disease, edema, ascites, nephritis, and hepatic cirrhosis. In all of his work he states that he has relied on the Wagner benzidin test as the most sensitive and reliable method.

Groat,[10] in 1913, published in the *Journal of the American Medical Association* a benzidin test, nearly as simple as the Wagner method, in which the feces were suspended in water, and barium dioxide was used instead of hydrogen peroxide.

A slight variation of Wagner's test (Dudley Roberts' test) is now being placed on the market in a very usable form by Squibb and Sons. This is described in the *Journal of the American Medical Association,* September 16, 1916, as follows: "This consists of tablets each containing 5 grains of a trituration of benzidin, 1 part, and sodium perborate 20 parts, and glacial acetic acid (supplied in boxes containing 100 tablets in vials, and a bottle of glacial

acetic acid). A tablet is placed in a small saucer or other suitable container; to it is added a quantity of the material to be tested (a weak solution of the stool or stomach contents or urine), sufficient to wet thoroughly, but not to cover the tablet entirely, and then a drop or two of the acetic acid is allowed to fall on the tablet; if blood is present, the tablet will turn greenish blue, the extent of the coloration and the time of its development depending upon the amount of blood present. (To avoid contamination of the glacial acetic acid remaining in the bottle, care should be taken not to touch the tablet or specimen with the rod which is used to transfer the acetic acid for the test.)"

The cooperation of the attendants of a patient in the procuring of a stool specimen is perhaps more spontaneous if the physician leaves with them the means of easily obtaining it. Very good for this purpose is one of the ordinary wooden tongue depressors,—to be used as a spatula—and a small porcelain ointment or salve jar with metal screw cap,—as a container.

CONCLUSIONS.

The Wagner benzidin test for occult blood is recommended on account of its ease, rapidity, and comparative cleanliness in execution, and on account of its reliability.

As carried out, this benzidin test is not too delicate for clinical use.

A negative benzidin test is more reliable evidence of absence of blood than is a negative guaiac test.

Meat fibers, pus, and the usual drugs and foods ingested do not interfere with the reaction. The ordinary hospital diet, with meat, gives a negative reaction; but, if sufficient quantities of rare meat be taken, the stool reaction may become definitely positive.

BIBLIOGRAPHY.

[1]Barker, L. F.: Monographic Medicine, D. Appleton & Co., New York, 1916, iii, 426.
[2]Grunwald, H. F.: Zur Trage des Blutnachweisse in den Fäces, Zentralbl. f. inn. Med., 1907, 109.
[3]Dewis, John W.: Comparative Tests for Occult Blood in Gastric Contents and Feces, with Especial Reference to the Benzidin Test, Boston Med. and Surg. Jour., 1907, clvii, 169-178.
[4]Brandenburg, K.: Ueber die Reaction der Leukocyten auf die Guajaktinctur, München. med. Wchnschr., 1900, xlvii, 183-186.
[5]Adler, O., and R.: Ueber das verhaltengewisser organischer Verbindungen gegenuber Blut mit besonderer Berücksichtigung des Nachweises von Blut, Hoppe-Seqler's Ztschr. f. physiol. Chem., 1904, xli, 59-67.
[6]Schlesinger, E., and Holst, F.: Vergleichen de Untersuchengen über den Nachweiss von Minimalblutungen in den Faeces nebst einer neuen Modifikation der Benzidinprobe, Deutsch. med. Wchnschr., 1906, 1444-1447.
[7]Wagner, A.: Zum nachweis okkulter Blutungen in den Fäces, Zentralbl. f. Chir., 1914, xli, 1182-1184; Arch. f. Verdauungskr., 1914, xx, 552-555.
[8]Cowie, D. M.: A Comparative Study of the Occult Blood Tests; A New Modification of the Guaiac Reaction; Its Value in Legal Medicine. Am. Jour. Med. Sc., 1907, 408-423.
[9]Gregersen, J. P.: (Occult Bleeding) Ugeskrift for haeger, Copenhagen, lxxviii, 1260.
[10]Groat, W. A.: An Improved Clinical and Microchemical Test for Blood, Jour. Am. Med. Assn., 1913, lxi, 1897.

The Journal of Laboratory and Clinical Medicine

VOL. II. MARCH, 1917 No. 6

Editor-in-Chief: VICTOR C. VAUGHAN, M.D.
Ann Arbor, Mich.

ASSOCIATE EDITORS

DENNIS E. JACKSON, M.D. - - ST. LOUIS
HANS ZINSSER, M.D. - - - NEW YORK
PAUL G. WOOLLEY, M.D. - - CINCINNATI
FREDERICK P. GAY, M.D. - - BERKELEY, CAL.
J. J. R. MACLEOD, M.B. - - - CLEVELAND
ROY G. PEARCE, M.D. - - - - CLEVELAND
ROGER S. MORRIS, M.D. - - CINCINNATI
GERALD B. WEBB, M.D. COLORADO SPRINGS

Contents of this Journal Copyright, 1917, by The C. V. Mosby Company—All Rights Reserved
Entered at the Post Office at St. Louis, Mo., as Second-Class Matter

EDITORIALS

The Relation of Body Surface to the Basal Metabolism of the Body

THE observations that small animals require a greater amount of food than do larger ones of the same species, and that children demand a greater food supply than adults, has led to much speculation as to what determines the energy requirements of the body. Years ago Rubner furnished experimental data which apparently proved that the relative metabolism of all mammals when reduced to terms of surface area was almost alike. If the body surface bears a casual relationship to the energy requirements of the body, the rate of cooling must be the determining factor. It can hardly be that such a relationship, if it does exist, can be casual, for the amount of heat lost through the lungs is considerable, and Rubner's law fails to take consideration of this.

Nevertheless, for years the standard textbooks of physiology have taught that such a relationship exists, and that the metabolism of the resting individual is proportional to the surface area and not to the body weight. Recent investigations of the metabolism of the body under conditions which give the lowest possible rate of energy consumption (nasal metabolism) have awakened a new in-

terest in the application of Rubner's law. If such a law correctly expresses the relative metabolism, a fixed figure representing the caloric output per hour and per square meter of body surface for normal people should furnish a valuable index for comparison with figures obtained similarly in cases of patients suffering from pathological conditions.

The observations of a number of investigators indicate that the average basal metabolism of adults in normal health amounts to about forty calories per hour and per square meter of body surface. This figure represents the average basal metabolism found in a large number of determinations in which the surface area of the body was most carefully estimated, and serves as a normal control for practically all the recent work on metabolism in disease.*

While it is no doubt true that the metabolism of the body varies more nearly directly with the body surface than with the body weight in normal adults, in infants Benedict and Talbot[1] failed to confirm the law. Murlin and Hoobler[2] although not agreeing with the conclusion of the above authors, conclude that there is no good reason for estimating the food requirements of the infant on the basis of surface area rather than on the basis of weight.

Recently Benedict[3] has added some very interesting observations on the changes occurring in the metabolism of a man during a fast of thirty-one days. By adapting the principles[4] of body surface measurement so admirably worked out by DuBois and Du Bois,[5] he was able to compute the surface area of a man whom he previously had thoroughly studied during a long fast, from a series of photographs taken at regular periods during the period of starvation. The surface area thus obtained allowed him to recalculate the metabolism of the man in terms of surface area and calories per hour. He found that during the fast the metabolism decreased twenty-eight per cent. He believes that this furnishes proof of the inapplicability of the surface area law when applied to subjects with widely varying states of nutrition, and concludes that a normal figure obtained from a large number of healthy subjects does not furnish a correct standard for comparison with that obtained in cases where there is a marked emaciation as in diabetes.

In this individual he failed to find any relationship between heat production and surface area, and again affirms his belief that the active mass of protoplasmic tissue rather than the surface area is one of the greatest factors in determining the metabolic activity of an animal.

It may be as Benedict and Talbot suggest, that the active mass of protoplasmic tissue develops proportionally to the body surface, and that therefore in normal individuals the body surface, while in no way determining the metabolism of the body, affords a measure of that factor which does play the major role in determining the body requirements.

BIBLIOGRAPHY.

[1]Talbot: Am. Jour. Dis. Child., 1914, viii, 1.
[2]Murlin and Hoobler: Am. Jour. Dis. Child., 1915, ix, 81.
[3]Benedict: Am. Jour. Physiol., 1916, xli, 292.
[4]Du Bois and Du Bois: Am. Jour. Physiol., 1916, xli, 275.
[5]Du Bois and Du Bois: Arch. Int. Med., 1915, xv, 868.

*See editorial, Jour. Lab. and Clin. Med., Sept., 1916, i, 928.

—*R. G. P.*